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Effects of Waste Oil on the Survival and Reproduction of the American Flagfish, *Jordanella floridae*

STEVEN F. HEDTKE[1] AND FRANK A. PUGLISI

U.S. Environmental Protection Agency, Environmental Research Laboratory—Duluth, 6201 Congdon Boulevard, Duluth, MN 55804, USA

HEDTKE, S. F., AND F. A. PUGLISI. 1980. Effects of waste oil on the survival and reproduction of the American flagfish, *Jordanella floridae*. Can. J. Fish. Aquat. Sci. 37: 757–764.

Short-term lethality tests, tests on the 30-d survival of 1-d-old larvae, and a life cycle test showed that used crankcase oil adversely affected the survival and reproduction of the American flagfish, *Jordanella floridae*. Four-day LC_{50}'s for juveniles in static and flow-through tests were 36 200 μL/L and 9500 μL/L, respectively, for the water-soluble fraction (wsf) of a 10% oil–water mixture and 485 μL/L and 82.7 μL/L, respectively, for oil–water emulsions. The general descriptive characteristics of the test oil and the hydrocarbon composition and concentration of 13 metals were determined for the oil and its water-soluble fraction. The life cycle test began with embryos and continued through 45 d of spawning. Egg production was impaired by exposure to 3380 μL wsf/L. Tissue residue analyses indicated the uptake of lead, cadmium, and zinc at higher exposure levels. The 30-d LC_{50} for larvae exposed as embryos was >8100 μL wsf/L and for larvae unexposed as embryos was 755 μL wsf/L. The maximum acceptable toxicant concentration for the water-soluble fraction of used crankcase oil was estimated to fall between 325 and 930 μL/L. Based on the compounds identified in the water-soluble fraction, previous toxicity data, and tissue residue analyses, we speculate that zinc, lead, and cadmium probably contribute significantly to the toxicity of the waste oil tested.

Key words: waste oil, used crankcase oil, American flagfish, lethality, reproduction, embryo–larvae, zinc, lead, cadmium

HEDTKE, S. F., AND F. A. PUGLISI. 1980. Effects of waste oil on the survival and reproduction of the American flagfish, *Jordanella floridae*. Can. J. Fish. Aquat. Sci. 37: 757–764.

Des essais de létalité à court terme, essais de survie de 30 d de larves de 1 d et un essai sur le cycle biologique entier démontrent que l'huile de carter usée nuit à la survie et à la reproduction de la jordanelle de floride, *Jordanella floridae*. La CL_{50} après 4 jours de jeunes soumis à des essais en eau dormante et en eau courante sont de 36 200 μL/L et 9500 μL/L, respectivement pour la fraction soluble dans l'eau (wsf) d'un mélange 10% huile–eau et 485

[1]Present address: U.S. Environmental Protection Agency, Newtown Fish Toxicology Station, 3411 Church Street, Cincinnati, OH 45244, USA.

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μL/L et 82,7 μL/L, respectivement pour les émulsions huile–eau. Sur l'huile utilisée dans les essais et la fraction soluble dans l'eau, nous avons déterminé les caractéristiques descriptives générales de l'huile et la composition en hydrocarbures ainsi que la concentration de 13 métaux. L'essai couvrant le cycle biologique commença avec les embryons et se poursuivit jusqu'à 45 d de ponte. Une exposition à 3380 μL de wsf/L entrave la production d'oeufs. Des analyses de résidus tissulaires indiquent qu'il y a absorption de plomb, de cadmium et de zinc à des niveaux d'exposition plus élevés. La CL_{50} après 30 d de larves exposées comme embryons dépasse >8100 μL de wsf/L, alors que pour des larves non exposées au stade d'embryon est de 755 μL de wsf/L. On estime que la concentration maximale acceptable de toxique pour la fraction soluble dans l'eau d'huile de carter usée se situe entre 325 et 930 μL/L. Nous basant sur les composés identifiés dans la fraction soluble dans l'eau, sur des données de toxicité antérieures et sur des analyses de résidus tissulaires, nous émettons l'hypothèse que le zinc, le plomb et le cadmium contribuent probablement de façon significative à la toxicité de l'huile usée testée.

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POLLUTION by petroleum hydrocarbons has been associated in the past primarily with spectacular marine disasters. However, a significant source of oil spills is the disposal of waste oil in freshwater. Estimates of the waste oil input to the environment in the United States have ranged from 2.27 to 5.03 $\times$ 10^9 L yearly (Bernard 1971; Ostrander and Kleinert 1973; Anon. 1974; U.S. Environmental Protection Agency 1974). Approximately 15% of this discharge has been used annually to oil roads for dust control; as much as 70% of this oil is washed off the roadway as runoff (Freestone 1972). In one dust-control spraying incident, the deaths of horses and other animals as well as the illness of several human beings were attributed to toxic components present in the waste oil used (Carter et al. 1975).

In spite of the volume released, freshwater toxicological studies have not been conducted with waste oil. Since both short-term accidental spills and long-term chronic discharges of waste oil can be expected, the purpose of this study was to determine the short-term lethal concentration and the long-term effect of waste oil on the survival, growth, and reproduction of the freshwater American flagfish, *Jordanella floridae*. This fish was chosen because of its rapid development to maturity and the ease of conducting tests on its reproductive success. Four-day lethality tests with both the water-soluble fraction and an oil–water emulsion were conducted to determine potential lethal concentrations due to accidental discharges. Since much aquatic toxicological data are generated in static tests, static as well as flow-through lethality tests were conducted for comparison purposes. It was felt that the majority of long-term environmental exposures would be with the water-soluble fraction. Therefore, long-term tests were conducted with this fraction. Long-term measurements included survival during a 30-d exposure starting with embryos and 1-d-old fish, effects of growth and reproduction of fish exposed from embryos through 45 d of spawning, and 30-d survival of resultant offspring. In addition, the used crankcase oil used as the toxicant was chemically characterized since no previous data on its composition existed.

Materials and Methods

Lake Superior water was used as the dilution water in all tests. Temperatures in flow-through tests were maintained by mixing heated and ambient Lake Superior water, and in static tests by placing all tanks in a constant temperature water bath. All flow-through tests were conducted with a modified 2-L proportional diluter (Mount and Brungs 1967; Benoit and Puglisi 1973).

SHORT-TERM LETHALITY TESTS

Four-day lethality tests were conducted with fish reared in laboratory cultures and fed live nauplii and frozen adult brine shrimp. At the time of testing, all fish had a standard length of 1.6 cm and ranged in age from 30 to 40 d and in weight from 0.13 to 0.14 g. Tests were conducted at 25°C as this has been determined to be suitable for flagfish reproduction (Spehar 1976).

Tests were conducted in all-glass 20 $\times$ 30 $\times$ 30 cm tanks containing 13.7 L of test solution. Five concentrations plus controls were set up in duplicate with each concentration at one-half of the next highest concentration. The test containers were randomly arranged and in flow-through tests received ~10 tank volumes of toxicant solution per day. A combination of Gro-Lux and Duro-Test[2] fluorescent bulbs was used to provide a constant 16-h daylight photoperiod at 40 $\pm$ 51 m at the water surface. Organisms were held at test temperatures at least 2 wk before testing. Feeding was stopped 48 h before beginning a test and was discontinued for its duration. Ten organisms were placed in each container after the addition of oil resulting in loadings of less than 0.1 g/L. In most tests, mortalities were measured at 0, 1, 3, 12, and 24 h and daily thereafter. Death was determined by lack of movement upon gentle prodding, and dead organisms were removed when found. The LC_{50}'s calculated by the moving average angle method (Harris 1959; Finney 1971) were determined only in those tests where the mortality was 65% or higher in one concentration and 35% or lower in another. A test was considered invalid if more than 10% of the control organisms died during a test.

Dissolved oxygen and pH were measured at the beginning, middle, and end of each test; alkalinity and hardness were

[2]The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

measured at the beginning and end (American Public Health Association et al. 1971). In general, the addition of oil caused a slight lowering of pH and dissolved oxygen concentration, but had no effect on hardness or alkalinity. The pH in all tests was between 7.1 and 7.7. The dissolved oxygen concentration was between 60 and 100% saturation in all tests. To prevent the driving off of volatile fractions, none of the tests were aerated. Hardness and alkalinity for all tests ranged from 42 to 49 and 40 to 44 mg/L as $CaCO_3$, respectively.

Life Cycle Exposure

A life cycle toxicity test with *J. floridae* and the water-soluble fraction of used crankcase oil was conducted according to the basic procedures described by Spehar (1976). The life cycle exposure began with embryos and continued through 45 d of spawning by the resultant adults. Duplicate nominal concentrations of 1.0, 0.4, 0.16, 0.06, 0.03, and 0.0% by volume of the water-soluble fraction stock solution were tested. The glass test containers were $30 \times 60 \times 30$ cm for adults, capacity 42.5 L; and $30 \times 38 \times 30$ cm for larvae, capacity 10.6 L. A constant 16-h daylight photoperiod of 100 ± 15 lm measured at the water's surface and a toxicant flow rate of six test-container volumes per day were used.

Tests were initiated by incubating sufficient freshly spawned embryos in each test concentration to get 20 fry for each test chamber to carry through reproductive tests. After 30 d fish were thinned to 15 in each chamber. At 5 wk, two spawning substrates consisting of stainless-steel screen wrapped with blue yarn were placed in each chamber. When a male exhibited territoriality over each substrate, fish were thinned to two males and five or six females. There was occasional female mortality during the test but this appeared to be unrelated to toxicant exposure. At the highest concentration no sexual dimorphism was apparent in one test chamber. The fish in this chamber were thinned to 10 organisms. No sexual maturity was observed in this tank throughout the test.

Reproductive studies were continued for 45 d after initial spawning began. Each day, eggs deposited on spawning substrates were removed, counted, and tested for hatchability. Hatchability was determined by selecting 30–50 embryos from each spawn, placing them in oscillating egg cups, and incubating them in the test chamber until hatching was completed. The number of embryos that hatched was recorded. Fifteen or twenty 1-d-old larvae were then transferred to larvae chambers at the same test concentration. Survival was determined at 4 and 8 d and weekly thereafter.

At the beginning of the life cycle test additional embryos were incubated in each concentration and transferred as 1-d-old larvae to other test concentrations. These tests were run for 30 d to determine if exposure as embryos affected sensitivity as larvae.

Effects of toxicants were determined from changes in survival, growth, eggs produced per female, and egg hatchability. Significant differences were determined by performing an analysis of variance with Dunnett's test of significance ($P < 0.05$; Steele and Torrie 1960).

Metal tissue residues were determined in whole bodies of fish thinned from the life cycle test at various times. A wet digestion procedure followed by atomic absorption spectrophotometry (Leonard 1971) was utilized to measure cadmium, lead, and zinc which were found in high concentrations in the waste oil. Hydrocarbon residues were also measured but the results were inconclusive.

Temperatures were monitored daily and were maintained at 24 ± 1°C with the exception of 1 d during the 2nd wk when temperatures dropped to 18°C for 6 h. Dissolved oxygen concentrations were measured in each test container three times a week and ranged between 65 and 95% saturation in all tanks throughout the test. Hardness, alkalinity, and pH were measured in four test containers each week and ranged from 43 to 48 mg/L as $CaCO_3$, 41 to 45 mg/L as $CaCO_3$, and 7.2 to 7.8 units, respectively. No change due to toxicant concentration was observed.

Toxicant Preparation and Measurement

The waste oil tested was used crankcase oil obtained from the Arrowhead Refinery Company of Duluth, Minnesota. Because waste oils may differ in their hydrocarbon and metal constituents, all oil obtained was from one batch and was stored in sealed oil drums.

Emulsified oils were prepared by blending oil and water at the required ratios in a Waring Blendor for 2 min. In static tests the toxicant solution was prepared at the beginning of the test. For flow-through tests, fresh oil was blended with dilution water each dilution cycle to form a new stock solution each time. This was subsequently dosed into the diluter on the following cycle.

The levels of emulsified waste oil in flow-through tests were monitored by fluorescence as determined on an Aminco-Bowman spectrophotofluorimeter. For each test at least two sets of samples were collected from each concentration plus a duplicate from one test container or from a duplicate concentration. Samples were extracted 1:1 with methylene chloride and, after the aqueous phase was removed, were dried with solvent-washed anhydrous sodium sulfate. The fluorescence of the extract was measured at a peak emission of 360 nm when excited at 300 nm.

The stock solution for the water-soluble fraction was created by recirculating water through an oil layer (10% oil – 90% water) for 18 h and allowing the resutlant mixture to separate for 6 h. Six 45-L glass carboys were connected in series with the water pumped from the bottom of one carboy to the top of the next by a small water pump connected in a line. A *t*-tube was placed on the end of the inlet tube to cause a gentle swirling of the oil and to prevent channeling of the water throughout the oil. After mixing and separation, the water-soluble fraction was drained into a Marriott system for delivery to the diluter. Because of the volume of toxicant required, it was necessary to prepare a new stock solution daily for short-term tests and weekly for the life cycle test.

The percentage of dilution of the used crankcase oil water-soluble fraction in flow-through tests was monitored by measuring lead with flameless atomic absorption spectrophotometry. Lead is a major contaminant of used crankcase oil because of the organometallic additives in lubricating oil as well as that picked up during engine wear (Supp et al. 1973; U.S. Environmental Protection Agency 1974). By measuring lead in the test chambers and in the water-soluble fraction stock solution, it was possible to determine the percentage of dilution of the water-soluble fraction present in the test containers.

Twice weekly, samples for lead analysis from each test container and the stock solution were collected in polyethylene bottles from mid-depth and preserved with concentrated perchloric acid. At least once a week duplicate samples were collected from a randomly selected container.

TABLE 1. Concentrations of the water-soluble fraction of used crankcase oil determined from measured lead concentrations used in test containers for the *Jordanella floridae* life cycle test.

	Measured lead concn.			μL/L stock solution based on $\bar{X}$ lead concn. (avg.)
	$\bar{X} \pm$ SD (μg/L)	Range (μg/L)	No. of analyses	
	37.0±8.3[a]	22.7–56.0	23	7920 (8100)
	38.7±8.5	24.8–55.1	21	8290
	16.0±3.9	7.33–23.4	22	3430 (3380)
	15.5±4.2	7.22–23.8	30	3320
	4.50±1.94	2.68–11.8	22	960 (930)
	4.22±1.47	2.52–8.42	21	900
	1.56±0.47	1.03–2.65	21	330 (325)
	1.48±0.35	0.98–2.54	20	320
	0.77±0.34	0.30–1.97	22	160 (155)
	0.71±0.24	0.32–1.29	21	150
	0.19±0.19	0.01–0.64	23	—
	0.15±0.14	0.01–0.60	21	—
Stock solution	4670±750	3440–6610	30	

[a]Each pair represents duplicate concentrations.

Measured levels for the life cycle test are reported in Table 1.

In all tests recovery of lead-spiked samples compared to aqueous standards was 90% or greater, replicate samples from the same test container agreed 95% or greater, and duplicate test containers agreed 90% or greater.

Characterization of the Waste Oil and its Water-Soluble Fraction

Because little previous knowledge on the composition of the waste oil tested was available, the used crankcase oil and the water-soluble fraction of that oil were analyzed. The organic components of the crankcase oil and its water-soluble fraction were characterized by separating the hydrocarbons from water with XAD-2 resin columns and subsequently into volatiles, saturated hydrocarbons, aromatic hydrocarbons, polar compounds, and solids by means of a silicic acid column. Analyses were conducted with gas chromatography mass spectrometry (GC/MS) according to methods previously described by Hedtke et al. (1974). The water-soluble fraction was also examined by gas–liquid chromatography (GLC) to estimate total saturated hydrocarbon concentration. Using the saturated hydrocarbon fraction separated from the waste oil by a silicic acid column as a standard, the concentration of total saturated hydrocarbons in the water-soluble fraction stock solution was determined. Additional analyses on the crankcase oil were performed by the Bartlesville Energy Research Center, U.S. Bureau of Mines. Included were general descriptive characteristics of lubricating oils and 13 metals. Metal concentrations in the water-soluble fraction were measured by atomic absorption again as described by Hedtke et al. (1974).

Results and Discussion

Toxicant Characterization

The chemical characterization of the crankcase oil and its water-soluble fraction (Table 2) indicated the oil was generally similar to eight other used lubricating oils analyzed by the U.S. Bureau of Mines (M. L. Whisman, Bartlesville. Energy Research Center, Bartlesville, OK 74003, 1973, unpublished data). Exceptions were a slightly lower calcium concentration and flash point and high percentage of sulphur and

TABLE 2. Characteristics of the used crankcase oil and its water-soluble fraction.

Item	Oil	Water-soluble fraction[a]
Inspection tests for lubrication units		
Sp gr (15.6/15.6°C)	0.906	—[b]
Viscosity		
SUS 37.8°C	290	—
SUS 99°C	60.9	—
Viscosity index	170	—
Carbon residue (%)	4.3	—
Flash point (°C)	58.5	—
Nitrogen (%)	0.090	—
Sulfur (%)	0.54	—
Antifreeze (%)	<0.1	—
Saponification number	14.5	—
Gasoline dilution	3.2	—
Total acid number	5.91	—
Total base number	2.06	—
Pentane insolubles	3.2	—
Coagulated pentane insolubles (%)	11.1	—
Benzene insoluble	1.45	—
Coagulated benzene insoluble	1.85	—
Solids (%)	15.6	—
Metals (μg/g for oil, μg/L for water-soluble fraction)		
Aluminum	15	<100
Copper	18	<100
Iron	220	<100
Lead	18 500	4 400±200[c]
Nickel	—	<100
Silicon	17	—
Antimony	6	—
Vanadium	—	—
Sodium	59	—
Barium	177	—
Calcium	688	—
Zinc	1 360	16 000±1000
Magnesium	410	—
Mercury	—	<0.1
Cadmium[d]	—	60±2
Molybdenum[d]	—	<200
Cobalt[d]	—	0.4±0.4
Arsenic[d]	—	<300
Selenium[d]	—	<100
Chromium	—	<140
Manganese	—	<100

TABLE 2 (*Concluded*)

Item	Oil	Water-soluble fraction[a]
Organics (+ indicates present but not quantified)		
Saturated hydrocarbons (%)	76.6	3.2
Aromatic hydrocarbons (%)	4.1	14.5
C_2 alkyl benzene	—	+
C_3 alkyl benzene	+	+
C_4 alkyl benzene	+	+
C_5 alkyl benzene	+	+
Ethylbenzene	+	—
Naphthalene	+	+
Methylnaphthalene	+	+
C_2 alkyl naphthalene	+	—
C_3 alkyl naphthalene	+	—
Phenanthrene	+	—
Methylphenanthrene	+	—
C_2 alkyl phenanthrene	+	—
C_3 alkyl phenanthrene	+	—
Dihydrodimethylphenanthrene	+	—
Dihydrodimethylindene	+	—
Diphenylmethane	+	—
Ethylstyrene	+	—
Dimethylindene	—	+
Polar compounds (%)	3.8	82.0
Volatile hydrocarbons (%)	3.2	—
C_2 alkyl benzene	+	—
C_3 alkyl benzene	+	—
C_4 alkyl benzene	+	—
C_5 alkyl benzene	+	—
Naphthalene	+	—
Methylnaphthalene	+	—
C_2 alkyl naphthalene	+	—

[a]Analysis of one test solution only. Lead concentrations in solutions prepared weekly for 30 wk were 4670 ± 750.

[b]Analyses not conducted or none detected.

[c]Mean multiple analyses ± standard deviation.

[d]Does not necessarily include contribution from a highly volatile fraction.

lead concentration. In the water-soluble fraction there was 3.2% saturated hydrocarbons compared to 76% in the oil and 14.5% aromatics compared to 4.1% in the oil. The concentration of total saturated hydrocarbons in the water-soluble fraction was found to be 11.6 mg/L. No chlorinated compounds were found. The presence of organo-lead compounds was suspected because of the removal of greater than 90% of the lead in the water-soluble fraction by the XAD column. More than 90% of the lead, zinc, and cadmium was in the dissolved fraction, which was operationally defined as those components passing through a 0.45-μm membrane filter.

SHORT-TERM LETHALITY TESTS

The calculated 96-h LC_{50}'s (and confidence intervals) for the water-soluble fraction (wsf) of waste oil in static and flow-through tests were 36 200 μL wsf/L (27 700 – 47 700 wsf/L) and 9500 μL wsf/L (6400–17 800 μL wsf/L), respectively. Ninety-six-hour LC_{50}'s for oil–water emulsions in static and flow-through tests were 485 μL oil/L (no confidence interval possible) and 82.7 μL oil/L (64.9–110 μL oil/L), respectively.

The short-term lethality tests indicated that flow-through tests were more toxic than static tests and emulsified oil was more toxic than the water-soluble fraction. Static tests were likely to be less toxic than flow-through due to the changes in chemical nature and toxicity of oil with time. Oils modified by aging have been shown to be less toxic than fresh oil (Morrow 1973; Templeton et al. 1975). In the flow-through tests, fresh oil was intermittently added throughout the test. The increased toxicity of emulsified oils could have been caused by increased oil in the water column, by a coating of the gills, and by a more rapid release of the soluble components due to the increased surface area for oil–water contact.

The greater toxicity of emulsified oils suggests that if long-term tests had been conducted with oil–water emulsions, effects might have been found at lower levels than with the water-soluble fraction. As stated previously, however, it was felt that while emulsions are likely to occur on a short-term basis, long-term exposures are more likely with the water-soluble fraction.

LONG-TERM TESTS

Exposure of flagfish to the water-soluble fraction of used crankcase oil during the life cycle test resulted in significantly ($P < 0.05$) lower egg production per female at 3380 μL wsf/L (Table 3). No difference was found at 930 μL wsf/L. In general, growth and embryo hatchability were not affected by exposure to oil at the concentrations tested. In addition, there was no significant larval mortality at any concentration when hatched and exposed at the same concentration (Table 4); the 30-d LC_{50} was greater than 8110 μL wsf/L. However, for those larvae hatched in control water and transferred as 1-d-old fish to various exposure concentrations, the 4-, 8-, and 30-d LC_{50}'s calculated from the data in Table 4 were 1660, 1000, and 755 μL wsf/L, respectively. No statistically significant toxicant related difference ($P < 0.01$) was found between the 30-d survival of second-generation larvae which were spawned, hatched, and exposed to the same concentrations at their respective parental exposure levels where spawning was adequate for testing. Mean survival ranged from 62.5 to 70.4%. No tests were possible at 8110 μL/L since spawning was insufficient to obtain adequate embryos for testing.

Whole body tissue residue levels for lead, cadmium, and zinc were higher at 3380 μL/L and 8110 μL/L than at 155 μL/L and in the controls (Table 5). There was little difference in residues at the two sampling time intervals. Statistical treatment of the data was not possible because of the limited number of replicate fish available for analyses.

TABLE 3. Growth and reproduction of *Jordanella floridae* exposed to the water-soluble fraction of used crankcase oil.

Mean oil concentration (μL water-soluble fraction/L water)	Females[a]			Males			Total eggs produced	Eggs per female (total/no. females)	Hatchability		
	No. present	Mean weight (g)	Mean length (cm)	No. present	Mean weight (g)	Mean length (cm)			Mean % hatch	Range % hatch	No. of tests
8100	4[b]	0.43[c]	2.4[c]	4[b]	0.98	3.0	3	<1[c]	—	—	0
	5	0.86	3.1	2	1.31	3.4	53	11[c]	—	—	0
3380	4	0.85	2.8	3	0.86	3.0	110	27[c]	67	67	1
	4	1.23	3.2	2	2.92	4.5	5	1[c]	—	—	0
930	5	0.93	3.1	2	2.11	4.1	973	195	58.3	28–100	9
	3	0.86	3.0	2	2.01	4.0	603	201	61.7	43–76	3
325	5	1.08	3.2	2	1.61	3.7	2070	415	60.4	46–89	15
	5	1.44	3.5	2	1.50	3.6	1420	285	59.0	32–82	9
155	6	1.02	3.2	2	1.77	4.0	1070	179	68.6	38–92	11
	5	0.90	3.1	2	1.97	3.9	827	165	57.3	38–76	6
0 (control)	6	1.10	3.4	2	2.15	3.8	1960	326	46.5	18–96	8
	5	0.96	3.2	2	2.14	4.2	1160	232	50.8	28–76	5

[a]Each pair represents duplicate tanks.
[b]Fish never reached sexual maturity.
[c]Significantly different from control value $P < 0.05$.

The long-term effects on survival and reproduction occurred at substantially lower concentrations than in the short-term lethality tests with juvenile fish. The lowest concentration affecting reproduction was 3380 μL wsf/L. The most sensitive test was the 30-d survival of larvae unexposed as embryos. The 30-d survival of larvae unexposed as embryos was significantly lower at 930 μL wsf/L (47.5% survival). The maximum acceptable toxicant concentration (MATC, Mount and Stephan 1967) or the highest concentration where there was no affect on survival, growth, or reproduction for the water-soluble fraction of a 10% mixture of used crankcase oil and water was, therefore, between 325 and 930 μL wsf/L.

The sensitivity of the embryo–larvae stage has been extensively reviewed previously (McKim 1977). Of the 56 tests included in this review, 82% of the time embryo–larvae or early juvenile exposures were identical with, or more sensitive than, other life cycle effects. The author states that while most of the data involves single toxicants, complex mixtures should give similar results. In the present test, larvae exposed as embryos

TABLE 4. Percentage survival of 1-d-old *Jordanella floridae* at various concentrations of the water-soluble fraction of used crankcase oil.

Embryo exposure (μL water-soluble fraction/L)	Larval exposure (μL water-soluble fraction/L)	Mean percentage survival[a]		
		4 d	8 d	30 d
0 (control)	8110	0.0	0.0	0.0
	3380	13.4	3.4	3.4
	930	81.7	70.9	47.5
	325	100.0	90.0	86.7
	155	92.5	90.0	90.0
	0	100.0 (4)	91.7 (4)	88.8 (4)
155	3380	0.0	0.0	0.0
	155	100.0	97.5	97.5
325	8110	2.5	2.5	2.5
	3380	—[b]	—	40.0
	325	100.0	97.5	95.0
930	8110	86.7 (1)	86.7 (1)	86.7 (1)
	3380	100.0 (1)	100.0 (1)	100.0 (1)
	930	100.0	97.5	85.0
	155	100.0	95.0	95.0
3380	8110	100.0 (1)	100.0 (1)	95.0 (1)
	3380	100.0	92.5	92.5
	325	97.5	92.5	92.5
8110	8110	97.5	82.5	82.5
	930	100.0	97.5	97.5
	0	100.0	97.5	92.5

[a]Mean of two tests with 15 fish per test unless number of tests otherwise indicated in parentheses.
[b]Observation not made.

TABLE 5. Metal residues in whole *Jordanella floridae* tissues after life cycle exposure to used crankcase oil.

Oil concn. (μL water-soluble fraction/L)	Exposure time (days)	Metal residue[a] (μg/g dry weight)		
		Lead	Cadmium	Zinc
0 (control)	30	<1.3	0.11	119
	75	1.4[b]	0.30[b]	99[b]
155	30	1	0.15	146
	75	1.3[b]	—	102[b]
3380	30	25	1.1	187
	57	22	0.9	143
8110	30	40	1.2	256
	64	50	1.3	291

[a]Average of at least duplicates of a two-fish composite.
[b]Based on a single two-fish composite.

were not affected by the waste oil concentrations tested and, therefore, were not as sensitive as the reproductive stage of the life cycle. However, tests with larvae not exposed as embryos were more sensitive than any other test. Thus, the evaluation of the sensitivity of embryo–larval tests may be affected by the stage of development during initial toxicant exposure.

The difference in 30-d LC_{50}'s between larvae exposed as embryos (>8110 μL wsf/L) and unexposed as embryos (755 μL wsf/L) suggests that acclimation occurred during the embryo stage. Additional evidence of acclimation was found with larvae exposed to oil at various concentrations as embryos and transferred upon hatching to other concentrations. At the lower concentrations (155 and 325 μL/L) the acclimation to waste oil allowed larval survival at the hatching concentrations. However, substantial mortality occurred when larvae were exposed to higher concentrations. Larvae exposed as embryos to concentrations of 930 μL/L and higher survived for 30 d at all tested concentrations. This implies an embryo-exposure threshold for acclimation to the range of concentrations tested. Other studies with zinc have also found acclimation due to exposure during the embryo stage (Pickering and Vigor 1965; Singley et al. 1974; Spehar 1976; Spehar et al. 1978). This apparent acclimation during the embryo stage may also have affected other end points of effect. Other life cycle effects might have occurred at lower concentrations than those found if the test started with 1-d-old larvae rather than embryos. Spehar (1976) found with zinc that the growth of female flagfish was affected at lower levels when started as 1-d-old larvae compared to those started as embryos.

A review of the identified compounds in the water-soluble fraction, previous toxicity data, and tissue-residue analysis suggests that the metals zinc, lead, and cadmium probably contribute significantly to the toxicity in the tests reported here. A decrease in embryo production by *J. floridae* in soft water has been reported by Spehar (1976) at concentrations of zinc between 139 and 267 μg/L. The 30-d $LC_{50's}$ calculated from Spehar's data were 64 μg/L for fry not exposed as eggs and 171 μg/L for fry exposed as eggs. Concentrations of up to 180 μg/L zinc were found in the higher oil concentrations in the present study. Cadmium concentrations of 8 μg/L (Spehar 1976) and lead concentrations of 62.5 μg/L (L. Anderson, U.S. Environmental Protection Agency, Environmental Research Laboratory—Duluth, 1976, personal communication) reduced flagfish reproduction in soft water. The highest tested oil concentration contained 0.5 μg/L cadmium and 38 μg/L lead. The effect of combinations of all three of these metals, however, has not been determined. In addition, the toxicity of organometallic compounds is largely unknown. From a life cycle test with *Pimephales promelas* and copper, zinc, and cadmium in hard water, Eaton (1973) concluded that the toxicities of the individual components were not strictly additive when mixed. Spehar et al. (1978) found that the toxicity of a mixture of cadmium and zinc to flagfish egg production was greater than for the individual metals but statistical significance could not be demonstrated. Effects on larval survival were attributed primarily to zinc in that same study. It is likely that the long-term effects of used crankcase oil are a result of a combination of a number of the components of the oil.

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Stress Responses to Transportation and Fitness for Marine Survival in Coho Salmon (*Oncorhynchus kisutch*) Smolts[1]

J. L. SPECKER AND C. B. SCHRECK

Oregon Cooperative Fishery Research Unit,[2] Oregon State University, Corvallis, OR 97331, USA

SPECKER, J. L., AND C. B. SCHRECK. 1980. Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Can. J. Fish. Aquat. Sci. 37: 765–769.

Smolting coho salmon (*Oncorhynchus kisutch*) were transported experimentally at low and high densities (12 and 120 g/L) for short and long periods (4 and 12 h). Because smolts can be transplanted directly to seawater, half of the fish in each treatment were transported to tanks containing seawater and half to tanks containing freshwater. Plasma corticosteroids and glucose were elevated at unloading in all groups, and corticosteroids were still above the resting level 24 h later. Potential smoltification indicators such as plasma thyroxin concentration and gill (Na^+/K^+)–ATPase activity were not affected by transportation. Increased corticosteroids were correlated to increased mortality in transported salmon compared to acclimated control fish when subjected to a bioassay of stress — severe confinement. The various transport regimens did not cause increased expression of latent bacterial kidney disease. In a further experiment, smolting coho salmon were released into a stream after transport for different durations. The number of fish migrating on the 1st d appeared to be inversely related to the degree of "stress" en route, but the movements of all groups were similar thereafter. We concluded that transportation caused stress in the fish regardless of hauling regimen; that increased corticosteroids may have potential as indicators of reduced performance capacity; that the greatest stress occurred during loading and the first few hours en route; and that transported smolting coho salmon seem to be equally fit for entry into freshwater or seawater.

Key words: coho salmon, stress, transportation, seawater entry, corticosteroids

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Des saumons coho (*Oncorhynchus kisutch*) en voie de smoltification furent transportés expérimentalement en lots de faibles et de fortes densités (12 et 120 g/L) sur de courtes et de longues périodes (4 et 12 h). Comme les molts peuvent être transplantés directement en eau de mer, la moitié des poissons de chaque lot furent placés dans des réservoirs contenant de l'eau de mer et l'autre moitié, dans des réservoirs contenant de l'eau douce. Chez tous les groupes, les corticostéroïdes et le glucose plasmatiques étaient élevés au moment du déchargement et, 24 h plus tard, les corticostéroïdes étaient encore à un niveau supérieur au niveau de repos. Le transport n'affecte pas les indicateurs de smoltification potentielle, tels que concentration de thyroxine plasmatique et activité de (Na^+/K^+)–ATPase branchiale. Il y a corrélation entre élévation de corticostéroïdes et accroissement de mortalité chez les saumons transportés, comparativement à des poissons témoins acclimatés, lorsque soumis à un essai biologique de stress — réclusion rigoureuse. Les divers régimes de transport ne sont pas la cause d'expression accrue de la maladie bactérienne latente du rein. Dans une autre expérience, nous avons mis en liberté dans un cours d'eau des saumons coho en voie de smoltification après transport de diverses durées. Le nombre des poissons émigrant le premier jour semble inversement relié au degré de « stress » en route, mais par la suite, les groupes se déplacent de la même manière. Nous concluons que le transport cause un stress chez les poissons, quel que soit le régime de transport; que l'élévation des niveaux de corticostéroïdes peut être un indicateur de capacité de performance réduite; que le stress maximal se produit au moment du chargement et pendant les quelques premières heures de route; et enfin que les saumons coho en voie de smoltification transportés semblent être également aptes à être libérés en eau douce ou en eau salée.

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JUVENILE anadromous salmonids are transported for various reasons and this causes mortality that may be directly attributable to transportation (Ayles et al. 1976). The primary responses to handling and confinement, as perhaps incurred by fish during transportation, include increased levels of circulating catecholamines (Mazeaud et al. 1977) and corticosteroids (Mazeaud et al. 1977; Strange et al. 1977, 1978). Secondary effects of handling include fluctuations in blood chemistry, such as hyperglycemia, and indications of osmoregulatory dysfunction (Wedemeyer 1972). Such physiological changes in transported fish could alter their capacity to tolerate any second stressing agent (e.g. those encountered after liberation into the wild) or alter their ability to adjust to direct entry into seawater.

The main objective of this study was to determine if transportation reduced the survival potential of smolting coho salmon (*Oncorhynchus kisutch*) by altering their ability to make the transition from freshwater to seawater. We defined a transported fish as being stressed if its capacity to perform in one of three bioassays were reduced compared to acclimated controls. The bioassays used to measure decreased performance included (1) increased percent mortality after 24 h in a severely crowded live-cage in seawater vs. freshwater, (2) increased expression of latent disease in seawater vs. freshwater, and (3) decreased percent outmigration following release into a stream.

We investigated (1) the effect that transportation has on potential smolt indicators such as plasma thyroxin levels and gill (Na^+/K^+)–ATPase activity in fish transported to seawater vs. those transported to freshwater, and (2) the relationships that duration in transit and hauling density have to the degree of stress experienced by transported coho salmon. Clinical indicators of stress have never been adequately correlated to reduced performance (see Schreck and Lorz 1979). Therefore, we evaluated the use of accepted clinical estimates of stress, such as plasma concentrations of corticosteroids and glucose, as indicators of actual reduced performance capacity.

Materials and Methods

The coho salmon smolts used in this study averaged 30 g and were obtained from Sandy Fish Hatchery, Oregon Department of Fish and Wildlife, Oregon. The four hauling regimens included low and high densities (12 and 120 g/L) and durations defined as short and long (4 and 12 h). The fish were transported in 200-L plastic cans that contained about 200 fish each; density was controlled by adjusting the amount of water in each can. Control groups were hauled at 12 g/L to Oregon State University's freshwater holding tanks in Corvallis and to the seawater holding tanks at the Marine Science Center in Newport on April 17, 1978. The fish used to assess the effects of transportation were transported to the seawater tanks on May 1, 1978, and to the freshwater tanks on May 8, 1978. They were held at less than 12 g/L in 1-m circular tanks with water flow at 4 L/min. Food was withheld the day before transport, but thereafter all fish were fed to satiation daily with Oregon Moist Pellets.

WATER QUALITY IN TRANSIT

Dissolved oxygen (Yellow Springs Instruments) remained near saturation (10.4 ± 3.9 mg/L) in all hauling cans. The ammonia concentration (Bausch and Lomb) remained between 2.0 and 3.4 mg/L in water in which fish were at a low density and between 6.3 and 10.6 mg/L in water in the high-density group. The pH (Beckman) remained between 6.4 and 7.5. Water temperatures were maintained at 11.5 ± 2.5°C, with a notable exception that on May 8, the temperature increased above 15°C during the first 4 h in transit before it could be reduced and maintained with ice.

EFFECTS OF TRANSPORTATION ON CORTICOSTEROIDS, GLUCOSE, THYROXIN, AND GILL (Na^+/K^+)–ATPASE ACTIVITY

Thirty fish per treatment (two replicate tanks; 15 fish per tank), including the acclimated controls, were sampled at the time of unloading and on days 1, 4, and 16 posttransport (PT). For additional comparison, 30 hatchery fish were sampled at the hatchery on the 2 d on which the fish were hauled. Fish were captured with minimal disturbance. Blood was collected from the severed caudal artery in heparinized pipettes, centrifuged, and frozen at −20°C until analyzed.

Corticosteroids were assayed in 15 μL of plasma according to Strange and Schreck (1978). Of the actual values determined by this assay, 80% were for cortisol and the rest were for other corticoids. Glucose was determined from a pooled ($n = 10$; 10 μL per fish) 100-μL plasma sample for each replicate, using the Harleco glucose reagent and Standard Set Kit (Gibbstown, NJ). Thyroxin was determined in 25 μL of plasma by using a radioimmunossay described by Dickhoff et al. (1978). The coefficients of variation for the radioimmunoassay of coho salmon plasma were 13.1% intraassay ($n = 10$) and 21.8% interassay ($n = 5$) between 50 and 57% binding in the assay. The average recovery of thyroxin added to plasma was 105% ($n = 4$).

Gill filaments were taken from exsanguinated fish ($n = 15$) at all blood sampling times from one replicate of each treatment group transported to seawater holding tanks. (Na^+/K^+–ATPase activity was determined by Dr W. Zaugg (National Marine Fisheries Service, Cook, WA).

BIOASSAYS OF STRESS

The potential stress imposed by the different hauling regimes and by direct entry into seawater was evaluated, in part, by subjecting the fish to a second stressing agent, severe and prolonged confinement. At the time of unloading, 12 fish from each treatment (replicated), including the acclimated control fish, were placed in a perforated, 1.5-L live-cage, and mortality was recorded.

Postmortem bacteriological analyses were performed on each dead fish for 6 wk following transportation. Following the protocol of the American Fisheries Society (1974), we could detect vibriosis, furunculosis, enteric red-mouth disease, and bacterial kidney disease.

To determine the ability of these coho salmon smolts to

migrate following release after transportation, we transported six groups of 150 smolts (average weight = 35 g) from Sandy Fish Hatchery, Oregon, on May 23, 1978 to Mill Creek, Polk County, Oregon, at three "levels" of stress in duplicate, representing controls at "minimum stress" (2.25 h en route at 12 g/L), and experimental fish at "moderate stress" (6 h en route at 120 g/L), and "high stress" (9 h en route at 120 g/L). These six groups had been distinctly cold-branded on April 28, 1978. The fish were placed in 200-L plastic cans at various times of the day so they could be liberated at the same time, but following different periods en route. Water temperature at the hatchery was 8.9°C; aeration was supplied by bubbling oxygen through air stones. The fish were released into Mill Creek, 5.6 km above a weir and downstream trap, at 17:45. Water temperature in the stream was 9.4°C.

Water Quality

Water quality in the hauling cans at the time of release of fish was consistent among groups, except for ammonia concentration, which averaged 0.8 mg/L, 8.4 mg/L, and 9.6 mg/L for the minimum, moderate, and high stress treatments, respectively. Dissolved oxygen ranged from 9.0 to 12.5 mg/L, temperature from 11.5 to 12.5°C, and pH from 6.4 to 6.7.

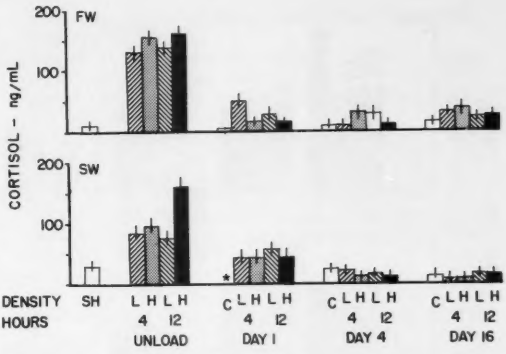

FIG. 1. Mean (±SE, vertical lines) cortisol concentrations (two pooled replicates per treatment; 10–15 fish per pool) in coho salmon after transportation at 12 g/L (low density (L)) or 120 g/L (high density (H)) for 4 or 12 h and released into freshwater (FW) or seawater (SW) holding tanks. Samples were taken at Sandy Fish Hatchery (SH) before treatment, at the time of unloading (UNLOAD) and at days 1, 4, and 16 after transportation. Acclimated controls (C) were also sampled, except when not available (*).

Results and Discussion

Transportation caused stress in coho salmon smolts under these experimental conditions. Initial moments of the capture–loading process may be the major effector of the stress response. All mortalities (1.9%) in transit occurred within 2 h after loading. No mortalities were observed thereafter that could be attributed directly to transportation. The importance of the capture element of stress has been recognized by Miles et al. (1974) in the muskellunge (*Esox masquinongy*) and by Barton et al. (1980) in rainbow trout (*Salmo gairdneri*).

Clinical Indicators of Stress

Clinical assessment of these transported smolts indicated that they were in a stressed condition. Plasma corticosteroid concentration was markedly elevated in all treatment groups when the salmon were unloaded (Fig. 1). Differences in corticosteroid levels between fish transported to seawater and to freshwater are probably the result of the temporary temperature increase experienced during transit by those fish taken to freshwater. Temperature is a critical factor in reducing mortalities in transported fish (Horton 1955) and probably contributed to the fact that all treatment groups had equally high corticosteroids.

Of the coho salmon transported to seawater, corticosteriods are higher in the fish from the high-density, long-duration treatment than in fish from other treatment groups. Water–ammonia levels were substantially higher in this treatment group. Although the data on en route mortality indicated that the first few hours, including loading, were the most stressful to the fish, these data from fish transported to seawater suggest that transportation continues to be stressful if the fish are maintained under adverse conditions.

Plasma corticosteroid levels returned to pretreatment concentrations within 24 h in the salmon placed in freshwater holding tanks, but remained slightly elevated in those placed in seawater. This slight difference could reflect a normal functional adjustment to osmoregulation in seawater, or additional stress of seawater. All groups exhibited "resting" levels by day 4 PT, a result in agreement with the findings of Strange and Schreck (1978).

Glucose concentration increased generally during transportation (Fig. 2), as might be expected after handling stress (Wedemeyer 1972). Hyperglycemia was not as pronounced as that found by Wedemeyer, probably because these fish had not eaten for 2 d before sampling at unloading. Carbohydrate metabolism also changed in Atlantic salmon (*Salmo salar*) as a consequence of transportation (Wendt and Saunders 1973).

Changes in Potential Smoltification Indicators

Transportation did not affect the concentration of circulating thyroxin. A transitory increase in circulating thyroxin following physical injury occurred in rainbow trout (Brown et al. 1978). We observed a general decrease through time, which is typical for coho salmon during this time of year (Dickhoff et al. 1978). Additionally, there was no trend in gill (Na^+/K^+)–ATPase activity among fish from the various treatment groups taken to seawater.

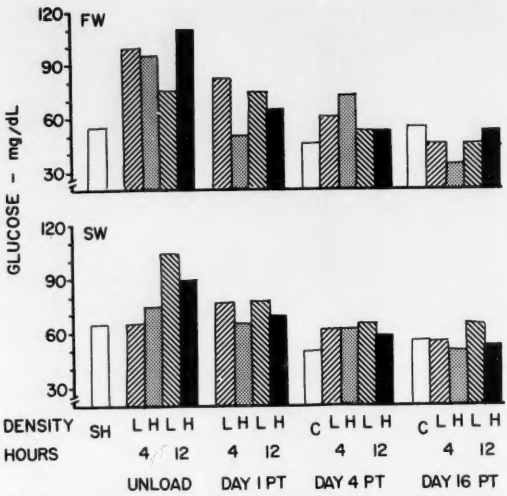

FIG. 2. Mean glucose concentrations (two pooled replicates per treatment; 10 fish per pool) in coho salmon transported at 12 g/L (low density (L)) or 120 g/L (high density (H)) for 4 or 12 h at unloading and 1, 4, and 16 d later. SH represents the mean glucose concentration in fish at Sandy Fish Hatchery on May 1 and May 8 before the treatment fish were transported to seawater (SW) or freshwater (FW), respectively.

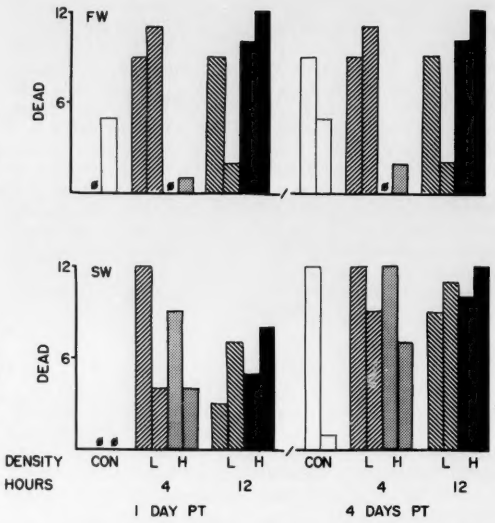

FIG. 3. Cumulative numbers of coho salmon that died by 1 and 4 d posttransport (PT) when 12 fish were subjected to severe confinement in live-cages after transportation at 12 g/L (low density (L)) or 120 g/L (high density (H)) for 4 or 12 h and released into freshwater water (FW) or seawater (SW) holdings tanks. Acclimated controls (CON) were placed in live-cages at the same time as those that had been transported. ϕ indicates that no mortalities were recorded.

PERFORMANCE OF TRANSPORTED SMOLTS IN BIOASSAYS OF STRESS

The increased mortality among transported coho salmon subjected to severe crowding compared to acclimated control fish supported the conclusion based on clinical tests that transported fish are stressed (Fig. 3). Many of the salmon in the 1.5-L crowding cages were dead within 24 h, whereas mortalities occurred in only one of the four acclimated control groups. Most fish were dead by day 4 PT; there were no differences among treatment groups. This bioassay of stress was found to be a fairly reliable indicator of degree of stress with chinook salmon (Strange and Schreck 1978). Coho salmon, however, persevere so much longer than chinook salmon that cause of death becomes confounded and the test loses sensitivity. Few fish died in the holding tanks during the first 6 wk PT, even though these fish were known carriers of bacterial kidney disease. Apparently, the transport regimen and elevated corticosteroids did not, in this instance, cause expression of disease.

The percentage of fish migrating was not affected by the different transportation treatments. All fish schooled in the pool at the release site within minutes after release. None of the fish appeared to be disoriented or stressed. No fish were seen during visual inspection of the pool on subsequent days. A total of 42% of all fish released were captured in the downstream trap in 8 d. However, the actual percentage of migrants may have been higher because the weir was not 100% efficient. On the day after release, it appeared as though the number of fish migrating was inversely related to the duration of transit ($n = 12$, 3, and 0 in 2.25, 6, and 9 h hauling duration, respectively). Thereafter, however, capture frequencies of all treatment groups were similar. No migration was evident on days 7 and 8 and the weir was removed on day 9. On day 4 and thereafter, many of the fish captured had opaque eyes, and one fish was found impinged on the weir. Consequently, the fish captured toward the end of the period may have entered the trap passively.

In summary, transportation reduced the ability of coho salmon smolts to tolerate a second stressing agent, severe and prolonged crowding. This reduced performance capacity and therefore stressed condition was correlated with clinical assessments of stress such as increased circulating corticosteroids and glucose. Transportation did not increase expression of disease, change migration-disposition, or affect potential smolt indicators such as circulating thyroxin and gill (Na^+/K^+)–ATPase activity. None of the tests in this experiment discerned differences in degree of stress imposed by different hauling regimes, although 98.9% of the fish that died in the first few hours en route were from the high-

density treatment group. Importantly, direct entry into seawater following transportation did not adversely affect the smolting coho salmon's performance in any of the bioassays of stress. This information suggests that transported smolting coho salmon are equally fit for entry into seawater or freshwater.

Acknowledgments

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Inhibition of Atlantic Salmon (*Salmo salar*) Hatching at Low pH

R. H. PETERSON, P. G. DAYE, AND J. L. METCALFE

Fisheries and Environmental Sciences, Fisheries and Oceans Canada, Biological Station, St. Andrews, N.B. E0G 2X0

PETERSON, R. H., P. G. DAYE, AND J. L. METCALFE. 1980. Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH. Can. J. Fish. Aquat. Sci. 37: 770–774.

Hatching of Atlantic salmon (*Salmo salar*) eggs was delayed or prevented if they were exposed to water of lowered pH (4.0–5.5) after eye pigmentation had developed. Hatching subsequently could be induced by returning eggs to normal pH levels (6.6–6.8). Perivitelline pH fell rapidly to near ambient levels when eggs were exposed to low pH. It is suggested that the observed effects on hatching were due to inhibition of the hatching enzyme, chorionase.

Key words: Atlantic salmon, eggs, pH, perivitelline fluid, chorionase

PETERSON, R. H., P. G. DAYE, AND J. L. METCALFE. 1980. Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH. Can. J. Fish. Aquat. Sci. 37: 770–774.

L'éclosion d'oeufs de saumon atlantique (*Salmo salar*) a été retardée ou empêchée par exposition à de l'eau de pH réduit (4,0–5,5) après formation de la pigmentation des yeux. L'éclosion a pu être provoquée par la suite en retournant les oeufs à des pH normaux (6,6–6,8). Quand les oeufs sont exposés à un bas pH, le pH périvitellin baisse rapidement à des niveaux presque ambiants. Nous suggérons que les effets observés sur l'éclosion sont dus à l'inhibition de l'enzyme d'éclosion, la chorionase.

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ACIDIFICATION of natural bodies of freshwater has become a concern in Scandinavia (Braekke 1976; Odén 1976), eastern Canada (Beamish et al. 1975; Beamish 1976) and the eastern USA (Cogbill 1976; Schofield 1976). Fish populations have been found to be particularly sensitive to acidification, which frequently causes a decline in reproductive success (Beamish 1974; Harvey 1979).

The pH levels lethal to eggs and alevins of several salmonid species have been determined (e.g. Carrick 1979; Daye and Garside 1977; Johanssen et al. 1977; Kwain 1975; Swarts et al. 1978). In these studies, pH was kept constant from fertilization to hatching. In several instances, it has been noted that the zona radiata appears "thicker" and is not shed readily by alevins hatching at low pH (Daye and Garside 1977; Johanssen et al. 1977). In these instances, the head and yolk sac of the alevins remained partially encapsulated for several days. No delay in hatching was observed. Runn et al. (1977) observed delayed hatching of perch (*Perca fluviatilis*) at pH 4.5–5.0.

In natural bodies of water, pH levels are rarely constant. For example, it has been observed that pH minima are frequently associated with the snowmelt (Elgmork et al. 1973; Haapala et al. 1975). Salmon egg and alevin mortalities have been observed in conjunction with these seasonal minima (Hagen and Langeland 1973; Leivestad and Muniz 1976). In this paper, we report on the hatching success of salmon eggs in advanced stages of development during exposure to low pH.

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Materials and Methods

Four experiments were performed to examine the effects of low pH on salmon egg hatching. In two experiments, eggs were examined very near hatching (450 degree-days) — one with running water (described below) and one a static test. A third (static) experiment utilized eggs in which eye pigmentation had just formed ("newly-eyed" eggs, 250 degree-days) and were about 1 mo from hatching. In a fourth (static) experiment, newly fertilized eggs were reared at various pH levels until hatching. In addition, a control experiment was performed using 3×10^{-5} mol/L Na_2SO_4 solutions to control for the addition of sulfate ions to the acidified solutions.

The Atlantic salmon eggs were of Waweig River (static tests) and Saint John River stocks. Those used in running water experiments (flow rate of 250 mL/min to produce a velocity of 1 cm/min past the single layer of eggs) were held at 8°C from fertilization. Those used in static experiments were reared under standard hatchery conditions until the experiments were begun, with temperatures decreasing from 10.5°C at fertilization to 6.5°C at the start of the first experiments. After experiments were begun, they were kept at temperatures of 7.5–8.0°C.

The source of water used in these experiments was Chamcook Lake, via the town of St. Andrews' water supply. Water was dechlorinated and filtered before use. The pH fluctuated from 6.4 to 7.1 during the experiments, and was normally 6.6–6.8. Water hardness was 13 mg/L as $CaCO_3$. While Na^+ and Cl^- levels were not measured, they were probably quite high, about 4.0 and 8.0 mg/L, respectively, which is typical of the water so near the coast

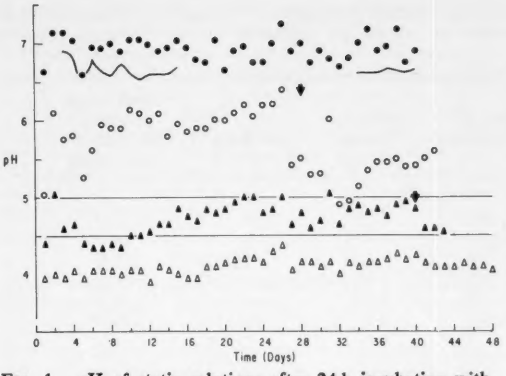

FIG. 1. pH of static solutions after 24 h incubation with eggs. (●) unadjusted tap water; (○) initial pH 5.0; (▲) initial pH 4.5; (△) initial pH 4.0. Straight lines indicate initial pH; wavy line shows ambient tap water pH. Arrows indicate occurrence of hatching.

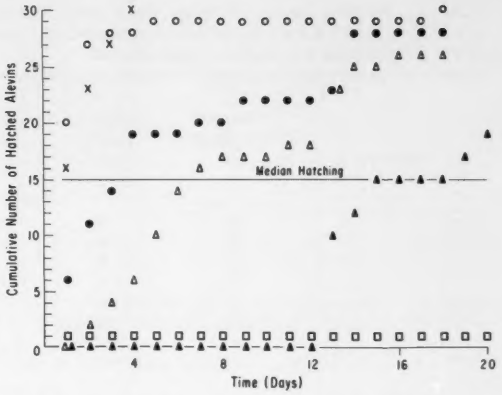

FIG. 2. Cumulative numbers of hatched alevins reared at various pH levels under running water conditions (30 eggs per test). (×) normal tap water (pH 6.6–6.8); (○) pH 6.0; (●) pH 5.5; (△) pH 5.0; (▲) pH 4.5; (□) pH 4.0.

(personal data from analyses of watersheds near St. Andrews).

Low pH levels for all experiments were attained by acidifying tap water with H_2SO_4, or HNO_3 (fourth experiment). In the running water experiment, the lowest pH (4.0) was maintained by a Radiometer TTTIC® controller regulating the flow of acid solution through a magnetic valve. Intermediate pH levels (4.5, 5.0, 6.0) were obtained by mixing tap water and water of pH 4.0 with the use of flowmeters. In the static experiments, solutions were changed daily. There was an initial ratio of 10–15 mL of solution per egg in the containers, but the ratio increased as eggs hatched and alevins were removed. One-litre plastic containers were used in all static tests, and all solutions were continuously aerated; pH was measured to ±0.05 pH units with a Radiometer model 22 pH meter calibrated over a suitable pH range.

The pH of the static solutions rose over the 24-h period until replaced by fresh solutions. The pH of unaltered tap water rose by 0.2–0.4 pH units (Fig. 1), whether or not eggs were present, and may be due to changing levels of CO_2 in the water supply which may have been supersaturated with regard to CO_2. The pH changes of the acidified solutions were of greater magnitude and were primarily due to substances released by the eggs. Preliminary experiments indicate that the substance responsible for the pH change is HCO_3^-, a conclusion based on the following observations: (1) the alkalinity of the static solutions increased by about 2 mg/L $CaCO_3$ over a 24-h period, (2) for solutions with initial pH values greater than 7.0, the pH decreases with time after eggs are introduced, while the pH increases with time in solutions when the initial pH is less than 6.5. There is very little change near pH 6.5 which is expected if the substance released were HCO_3^- with a pK of 6.5. The amount of change was inversely proportional to the ratio of solution volume to egg number. As hatching proceeded and alevins were removed, this ratio increased and therefore the pH changed less (Fig. 1, open circles and solid triangles, after hatching). Alevin length at hatching was measured to ±0.5 mm.

The pH of the perivitelline fluid of salmon eggs near hatching was measured with a micro-pH electrode imbedded in the tip of a 21-gauge hypodermic needle (Microelectrodes, Inc.®). The reference electrode was a micropipette filled with 3 mol/L KCl saturated with AgCl. A chloridized silver wire was inserted in the pipette; pH levels were recorded by an Orion model 407A specific ion meter. The egg was dried with absorbent tissue, then mounted in a humid chamber on a vertical piece of polyethylene tubing of slightly smaller diameter than the egg. The needle electrode was inserted into the egg near the embryo, as the perivitelline fluid was of greatest depth there. Care was taken not to touch embryonic tissue or the perivitelline membrane. The reference electrode was inserted through the same puncture. The electrode system was standardized before and after measurements on one to three eggs.

The criterion for egg mortality was death of the embryo (easily observed in these advanced stages of development) rather than yolk precipitation.

Results

When Atlantic salmon eggs just prior to hatching were transferred from normal tap water to water of pH 5.5 or less, hatching was delayed or prevented. In a running water system, the median hatching time was 3, 7, and 15 d longer at pH values of 5.5, 5.0, and 4.5, respectively (Fig. 2). Hatching was prevented at pH 4.0. The total numbers of alevins hatching were reduced at the lower pH levels. Total egg mortalities (includes mortality after partial hatching) were 0, 3, 30, 40, 37, and 97% at pH 6.8, 6.0, 5.5, 5.0, 4.5, and 4.0, respectively. Almost all of the egg mortality at low pH occurred long after hatching had been complete at normal pH. At pH 4.0, only one embryo died prior to 20 d after the start of normal hatching. The pH at which 50% mortality occurred as a result of delayed hatching was between 4.0 and 4.5.

TABLE 1. Hatching success of alevins reared under "constant" and "varied" pH regimes. The eggs were transferred from pH 6.6–7.0 to low pH levels at earliest eye pigmentation. "Constant" vs. "varied" pH regimes refer to period after development of eye pigmentation. n = number of eggs per test.

pH treatment	Time to 50% hatch (d)	Hatching period (d)	Normal alevins no. (%)	Deformed alevins no. (%)	Mortality no. (%)	Mean length of normal alevins at hatch (mm)	n
			"Constant" pH regimes				
6.6–7.0	28	3	12 (80)	3 (20)	0	17.9	15
6.6–7.0	30	4	10 (83)	2 (17)	0	18.2	12
5.0–6.2	28	6	8 (53)	2 (13)	5[a] (33)	17.9	15
5.0–6.2	30	4	10 (67)	1 (7)	2+2[a] (27)	17.8	15
4.5–5.0	34	9	8 (53)	1 (7)	3+3[a] (40)	19.8[c]	15
4.5–5.0	35	12	7 (47)	0	2+6[a] (53)	18.9[d]	15
4.0–4.2	—	—	—	—	4 (100)	—	4
4.0–4.2	—	—	—	—	4 (100)	—	4
			"Varied" pH regimes				
4.0 (29 d)→6.8	<1	<1	4 (80)	1 (20)	0	18.1	5
4.0 (33 d)→6.8	3	2	4 (80)	1 (20)	0	17.8	5
4.0 (38 d)→6.8	—	—	—	—	5 (100)	—	5
4.0 (41 d)→6.8	3	3	4 (80)	—	1 (20)	18.2	5
4.0 (47 d)→6.8	—	—	—	—	2 (100)	—	2
6.8 (29 d)→4.0 (11 d) ↓ 6.8	4 after return to 6.8	1	1 (33)	0	0	—	3[b]

[a]Partly hatched with head and part of yolk sac free, obviously deformed.
[b]Chorion was removed from remaining two eggs after 12 d back to pH 6.8 — curled up initially but appeared normal 11 d later.
[c]Differs significantly from all other mean lengths, $P < 0.05$.
[d]Differs significantly from first four mean lengths in table (ANOVA, Duncan's multiple range test, $P < 0.05$).

The phenomenon of delayed hatch was further investigated with static tests on newly eyed eggs (Table 1). The median hatching date was delayed by about 6 d at pH 4.5–5.0, and hatching did not occur at pH 4.0–4.2. Deformities were not increased at low pH and most egg mortalities either occurred well past the normal hatching time or with the alevin partially hatched. Growth appeared to continue for alevins past term, as those which were delayed were larger upon hatching. Hatching was induced by returning eggs past term from pH 4.0 to pH 6.8 for up to 10 d past time of normal hatching (Table 1, varied pH regimes). Beyond 10 d, hatching usually did not occur consistently after return to normal tap water (none at 10 d, 4 of 5 at 13 d, none at 19 d), even if hatching were imminent before exposing the eggs to low pH.

In another static experiment, with eggs near hatching when the experiment began (Table 2), exposure to a

TABLE 2. The effects of low pH on hatching success of salmon eggs. The eggs were incubated at pH 6.6–6.8 until hatching was imminent then exposed to low pH levels as indicated in the treatment column. In some instances, hatching was induced by returning the eggs to normal pH. n = number of eggs for each treatment.

pH treatment	Time to 50% hatch (d)	Hatching period (d)	Normal hatch no. (%)	Deformed hatch (%)	Hatching mortality no. (%)	Dead eggs	Mean length at hatch (mm)	n
6.6–7.0	1	2	20 (100)	0	0	0	18.8	20
4.0–4.2	—	—	0	0	2 (10)	18	—	20
4.0–4.2	—	—	0	0	3 (10)	27	—	30
4.0–4.2	5	1	1 (3.3)	0	11 (37)	18	20.0	30
4.0 (1 d)→6.8	1[a]	2	10 (100)	0	0	0	19.0	10
4.0 (1 d)→6.8	1[a]	2	9 (90)	0	1 (10)	0	19.3	10
4.0 (3 d)→6.8	2[a]	2	8 (80)	0	0	2	19.4	10
4.0 (3 d)→6.8	2[a]	2	7 (70)	0	1 (10)	2	19.7	10
4.0 (5 d)→6.8	2[a]	9	5 (50)	0	4 (40)	1	19.4	10
4.0 (7 d)→6.8	2[a]	3	2 (20)	1	5 (20)	2	20.0	10

[a]Days after return to pH 6.8.

TABLE 3. Effects of Na_2SO_4, on hatching of Atlantic salmon eggs.

Date (started Feb. 2, 1979)	Normal tap water			Na_2SO_4 added		
	pH	Temp. (°C)	No. hatched	pH	Temp. (°C)	No. hatched
Feb. 3, 1979	6.60	7.2	52/100	6.75	7.2	40/100
Feb. 4, 1979	6.60	8.1	100/100	6.70	7.2	88/100
Feb. 5, 1979	—	—	—	—	7.2	100/100

pH of 4.0–4.2 again prevented hatching (1 of 80 eggs hatching). Again, egg mortality occurred well after the termination of the normal hatching period, and returning eggs to normal pH resulted in rapid hatching, although the percentage of hatch declined to 50 and 20% after 5 and 7 d at low pH, respectively. Alevins delayed in hatching were larger, indicating continued in ovo growth. An increased percentage of unsuccessful hatching, resulting in dead, partially hatched alevins, also occurred in this experiment for eggs kept at low pH and for those reintroduced to normal pH after 5–7 d of exposure. There was no apparent effect of $SO_4^=$ on hatching time (Table 3).

In contrast to the delay in hatch occurring for eggs exposed to low pH levels after "eyeing," eggs reared at pH values of 4.5–5.5 from fertilization were not delayed relative to those reared at normal pH (Table 4). Eggs could not be reared at pH 4.0 from fertilization as they died in the earlier stages of development.

When eyed eggs were transferred from normal tap water to water of low pH, the pH of the perivitelline fluid fell rapidly (Fig. 3), approaching a steady state at about 8 h. For eggs transferred to pH 5.5, the final pH of the perivitelline fluid was 5.5. After transfer to pH 4.0, the perivitelline fluid was maintained at pH 4.4.

Discussion

Atlantic salmon eggs subjected to low pH during the later stages of development may be delayed in hatching or, if the pH is as low as 4.0, be prevented from hatching. These eggs are capable of hatching if returned to a higher pH (6.6–6.8) less than 8 to 10 d after the normal hatching time. It is well established that a hatching enzyme (chorionase) is secreted by

TABLE 4. Hatching times of alevins reared under static conditions at several pH levels from fertilization.

pH	No. of eggs	Time to 50% hatch (from fertilization) (d)	Degree-days to 50% hatch
6.8	68	90	533.0
5.5	63	88	517.1
5.0	53	88	511.9
4.5	53	91	543.3

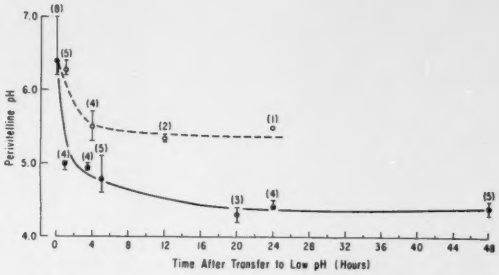

FIG. 3. Perivitelline pH of eggs after various incubation times in pH 5.5 (○) and pH 4.0 (●). Initial values are for eggs incubated in pH 6.6–6.8. Numbers of eggs measured given in parentheses. Bars indicate ranges.

the integument of the snout (Hayes 1942, 1949; Bell et al. 1969) to digest much of the zona radiata. The pH optimum of the hatching enzyme of rainbow trout (*S. gairdneri*) has been found to be near 8.5 (Hagenmaier 1974), with activity being reduced to 10% of the optimal rate at a pH of 5.2. We have shown that the pH of the perivitelline fluid falls to near that of the incubation medium within about 8 h. Since the hatching enzyme is secreted into the perivitelline fluid, we suggest that the action of this enzyme is inhibited at low pH, and can account for the effects on hatching observed here. Runn et al. (1977) found that the capsule of perch eggs was not degraded at low pH, also suggesting an inhibition of enzyme action. When the eggs are returned to pH 6.6–6.8, the enzyme activity increases and hatching can occur.

Eggs which had been at pH 4.0 longer than 30 d in Table 1 and longer than 3 d in Table 2 showed poor hatching success upon return to pH 6.8. The reason for this is unknown. It is possible that enzyme concentration in the perivitelline fluid had declined at this point.

A reviewer made one interesting suggestion that embryos exposed to low pH may be less active, hence may be less efficient at distributing the hatching enzyme throughout the perivitelline fluid. This may lead to less rapid digestion of the chorion. Embryos from all incubation pH levels were rhythmically moving their pectoral fins when perivitelline pH's were being measured. Frequencies were not determined, nor were systematic observations made of the more infrequent trunk movements. While we consider the lowered perivitelline pH the probable cause of delayed hatch, the question of reduced embryo movement should be more completely examined.

The hatching delay described above was not observed for eggs reared from fertilization at low pH, nor has any delay been found by others for eggs reared under similar conditions (Daye and Garside 1979; Johanssen et al. 1977). In contrast, Trojnar (1977) found hatching of brook trout eggs reared at low pH to be more rapid than those at higher pH values. It

would appear that some sort of adaptive process has occurred during early development at low pH which permits normal hatching.

The results presented here are of possible ecological significance. The pH minima of salmon streams often occur during the period of snowmelt in the spring (Gjessing et al. 1976; R. H. Peterson et al., 1978, personal observations). This low pH phase may coincide in some instances with the hatching period of salmon eggs (sometime in April). Therefore, delayed hatch could occur naturally if the depression in pH were great enough. High salmon egg and alevin mortality has been observed to occur during the snowmelt period in Scandinavia (Leivestad and Muniz 1976; Gjessing et al. 1976), although direct lethal effects of low pH have also been implicated in these instances.

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Genetic Differences and Environments of Mussel Populations in the Maritime Provinces

K. E. GARTNER-KEPKAY

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1

L. M. DICKIE AND K. R. FREEMAN

Department of Fisheries and Oceans, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

AND E. ZOUROS

Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1

GARTNER-KEPKAY, K. E., L. M. DICKIE, K. R. FREEMAN, AND E. ZOUROS. 1980. Genetic differences and environments of mussel populations in the Maritime Provinces. Can. J. Fish. Aquat. Sci. 37: 775–782.

Genetic differences among *Mytilus edulis* populations from four localities in Atlantic Canada were examined using electrophoretic techniques. On the basis of allele frequency distributions at four loci (LAP-1, PEPTIDASE-2, PGI, PGM), the localities fell into two pairs, paralleling a similar pairing on the basis of values of specific environmental parameters and their variability. Gene flow between environments could be eliminated as an explanation of gene frequency similarity in one of the pairs. The large heterozygote deficiencies appear not to be satisfactorily explained by the Wahlund effect, inbreeding, or "silent alleles," without the added influence of environmental selection. We conclude that the allele frequency patterns reflect differences in the average environmental characteristics among localities.

Key words: isoenzymes, blue mussels, genetic differences, environmental selection, *Mytilus edulis*

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Faisant appel à des techniques électrophorétiques, les auteurs analysent les différences génétiques parmi des populations de *Mytilus edulis* de quatre localités de la côte canadienne de l'Atlantique. D'après la distribution des fréquences des allèles à quatre loci (LAP-1, PEPTIDASE-2, PGI, PGM), les localités se groupent en deux paires, parallèlement à un semblable groupement en paires sur base des valeurs de paramètres environnementaux spécifiques et de leur variabilité. On a pu éliminer les échanges géniques entre environnements comme explication de la ressemblance de fréquence des gènes dans une des paires. Les fortes déficiences hétérozygotes ne semblent pas s'expliquer de façon satisfaisante par l'effet de Wahlund, la consanguinité ou les «allèles silencieux», sans l'influence supplémentaire d'une sélection environnementale. Les auteurs concluent que les modalités de fréquence des allèles reflètent des différences de caractéristiques ambiantes moyennes entre ces localités.

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THROUGHOUT its range, the blue mussel, *Mytilus edulis*, is exposed to large seasonal and daily environmental variations. Although its tolerance of environmental extremes enables it to colonize the littoral zone worldwide, it is sensitive to local conditions as is reflected in geographic variations in growth rate and population densities (Seed 1976). For these reasons, *M. edulis* is a species potentially suitable for monitoring marine coastal environments.

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To compare environments, Widdows (1978) has begun to develop stress indices based on the integration of physiological responses of individuals. Freeman and Dickie (1979) have explored the usefulness of growth and mortality parameters. In any such comparisons, however, it is necessary to determine the extent to which responses to environment are mediated genotypically or phenotypically.

Electrophoretic separation of isoenzymes in mussels has led to many studies of genetic variation among mussel populations. The variation at a number of gene loci over a broad environmentally diverse range has

been extensively reviewed by Levinton and Koehn (1976). Variations in allele frequencies are known in populations as widely separated as North America, Iceland, Ireland, and Australia, and have been ascribed to geographic isolation of breeding stocks. However, microgeographic variations of comparable magnitude have been demonstrated in estuaries (Koehn et al. 1976), and in the intertidal zone (Koehn et al. 1973), situations in which it might be expected that larval dispersal would have a homogenizing effect. Variation at the leucine aminopeptidase (LAP-1) locus gives an indication of the dynamic nature of this polymorphism. Milkman and Koehn (1976) have shown that the LAP-1^{94} allele frequency is size-dependent and can be observed to change over time. Others have shown its response to environmental gradients, particularly of salinity and temperature (Koehn and Mitton 1972; Koehn et al. 1976; Milkman et al. 1972; Lassen and Turano 1978; Levinton and Suchanek 1978). This genetic variation in the presence of local spatial and temporal environmental heterogeneity suggests that normal environmental perturbations may be exerting selection effects on cohorts which influence genetic constitutions on the sampling scale used. If this is true, the effect may have an important influence on the general usefulness of either physiological or production parameters as environmental indicators.

In this paper, we examine the variation in allele frequencies of four isoenzymes in mussels collected from natural populations at four locations in the Maritime Provinces. The population sampling was intended to complement the results of field experiments in progress in which the measurement of growth and mortality of mussels are being observed in situ and in reciprocal transfers among localities. In addition, the data may indicate the feasibility of using mussels in baseline studies to determine changes brought about by the effects of various uses of the environments in question.

Methods

Four localities were chosen for sampling — St. Margaret's Bay (SMB) and Bedford Basin (BB), two moderate-sized neighboring inlets on the Atlantic coast of Nova Scotia; Bideford River Estuary (PEI), an inlet of Malpeque Bay on the northern side of Prince Edward Island; and Apple River (BF), a small inlet located on the south side of Chignecto Bay at the head of the Bay of Fundy (Fig. 1). All localities have seasonally variable environments characteristic of temperate waters; however, environmental variability may differ from place to place depending on local conditions. Mussels were collected subtidally at SMB, BB, and PEI. Since mussel beds are generally confined to the tidal flats of Bay of Fundy, mussels were collected intertidally at this location.

Samples of between 300 and 400 mussels were collected from each of the four localities during the autumn of 1978. Individuals were picked from mussels distributed along 100 m of coastline and transferred to holding facilities in the Aquatron at Dalhousie University where they were kept alive in flowing seawater at 5–7°C until preparation for

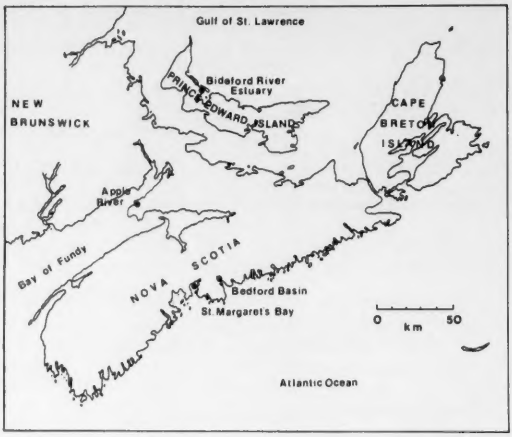

FIG. 1. Mussel sampling localities in the Maritime Provinces.

electrophoresis. One hundred animals between 30 and 45 mm in shell length from each source stock were selected for the electrophoretic experiments. The shells were cut open, and the flesh excised and individually homogenized. The homogenates were placed in a refrigerated Sorvall centrifuge at 2°C and centrifuged at 8000 rpm for 20 min. The supernatant was then quickly frozen at −75°C.

Horizontal starch gel electrophoresis using the discontinuous Poulik buffer system of Singh and Zouros (1978) was employed to determine genetic variability at four gene loci simultaneously. Fifteen animals from at least three of the four locations were represented on each zymogram. In addition, five marker animals, chosen in preliminary experiments to cover the entire range of mobilities, were included as a reference base for comparing allele compositions of test animals. Four isoenzymes were recorded — PGI (phosphoglucose isomerase), PGM (phosphoglucose mutase), LAP-1 (leucine aminopeptidase; also known as Aminopeptidase-1), and Peptidase-2 (formerly termed LAP-2). PGI and PGM are enzymes involved in the metabolic pathways for glycolysis and gluconeogenesis. LAP-1 and Peptidase-2 take part in protein metabolic reactions. These loci were chosen after preliminary experiments on the basis of their electrophoretic resolution and the clarity of response to staining techniques of Shaal and Anderson (1974). LAP-1 is a well-studied locus and four alleles have been observed by Koehn et al. 1976. LAP^{100} allele is rare and has not been observed in our samples.

Results

All of the gene loci examined are polymorphic and the alleles are designated according to their relative electrophoretic mobilities.

LEUCINE AMINOPEPTIDASE-1

LAP-1 shows three alleles common to all locations (designated $LAP\text{-}1^{98}$, $LAP\text{-}1^{96}$, $LAP\text{-}1^{94}$, after Koehn

TABLE 1. The allele frequencies for each isoenzyme observed in the four sampling locations.

	Allele frequencies					
Locality	LAP-1^{98}	LAP-1^{96}	LAP-1^{94}			
BF	0.49	0.35	0.16			
PEI	0.62	0.34	0.04			
BB	0.47	0.33	0.20			
SMB	0.37	0.29	0.29			
	PEPTIDASE-2^{110}	PEPTIDASE-2^{100}	PEPTIDASE-2^{95}	PEPTIDASE-2^{90}		
BF	0.32	0.35	0.20	0.13		
PEI	0.50	0.43	0.05	0.02		
BB	0.12	0.25	0.22	0.41		
SMB	0.11	0.25	0.32	0.32		
	PGI^{100}	PGI^{97}	PGI^{90}			
BF	0.32	0.38	0.30			
PEI	0.43	0.25	0.32			
BB	0.15	0.26	0.59			
SMB	0.09	0.21	0.70			
	PGM^{110}	PGM^{100}	PGM^{95}	PGM^{90}	PGM^{85}	PGM^{80}
BF	0.13	0.32	0.30	0.23	0.02	0.00
PEI	0.17	0.36	0.25	0.18	0.04	0.00
BB	0.10	0.23	0.21	0.35	0.09	0.02
SMB	0.06	0.21	0.22	0.37	0.09	0.05

et al. 1976). LAP-1^{98} is the most common allele in all locations, but the proportional frequency varies considerably from 0.37 in SMB to 0.62 in PEI (Table 1). LAP-1^{94} ranges from 0.04 in PEI to 0.29 in SMB. Figure 2a illustrates the allele frequency distributions for each location. Paired comparisons between localities were made using the chi-square test for heterogeneity (Sokal and Rohlf 1969). Results indicate significant differences among all paired comparisons except between BF and BB samples (Table 2).

PEPTIDASE-2

Four alleles of Peptidase-2 (Peptidase-2^{110}, Peptidase-2^{100}, Peptidase-2^{95}, Peptidase-2^{90}) were present in varying proportions in all locations. Relative frequencies of Peptidase-2^{110} range from 0.11 in SMB to 0.50 in PEI, while Peptidase-2^{90} varies from 0.02 in PEI to 0.41 in BB (Table 1). The plots of allele frequency distribution (Fig. 2b) illustrate the dominance of the slower migrating alleles in SMB and BB, and of the faster migrating alleles in BF and PEI. Chi-square values for paired comparisons show significant differences for all except the BB and SMB samples (Table 2).

PHOSPHOGLUCOSE ISOMERASE

Three alleles were detected at the locus for PGI (PGI^{100}, PGI^{97}, PGI^{90}), and were present at all four sampling locations. As shown in Table 1, the allele frequencies vary considerably between areas. PGI^{100} varies from 0.09 in SMB to 0.43 in PEI, while PGI^{90} ranges from 0.30 in BF to 0.70 in SMB. The allele frequency distributions among locations are plotted in Fig. 2c and illustrate, as in Peptidase-2, the dominance of less mobile alleles in BB and SMB, and more mobile alleles in BF and PEI. Chi-square values for the paired comparisons show significant differences among all paired comparisons except BB vs. SMB (Table 2).

PHOSPHOGLUCOSE MUTASE

Six alleles were detected for the PGM locus (PGM^{110}, PGM^{100}, PGM^{95}, PGM^{90}, PGM^{85}, PGM^{80}). PGM^{85} and PGM^{80} were rare alleles occurring at very low frequencies in all locations. The greatest differences in frequency among locations (Table 1) are found in PGM^{100} (0.21 in SMB to 0.36 in PEI) and PGM^{90} (0.18 in PEI to 0.37 in SMB). Figure 2d again shows the dominance of the less mobile allele (PGM^{90}) in SMB and BB and the more mobile

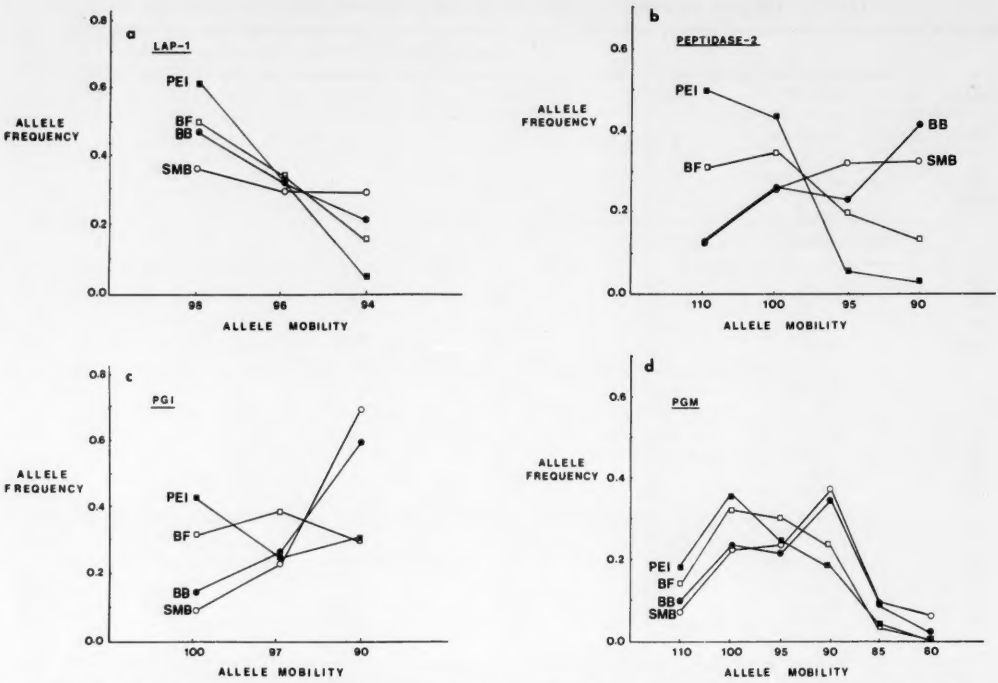

FIG. 2a–d. Graphical representations of allele frequency distributions for each locality. The *x*-axis is dimensionless and represents the assigned relative mobility values of alleles detected at each locus.

allele (PGM^{100}) in BF and PEI. Chi-square values for the paired comparisons suggest that the samples fall into two pairs, the BB and SMB samples being different from the BF and PEI pair (Table 2).

The use of Nei's (1972) index of genetic identity allows us to set up a matrix of average genetic similarity between the pairs of locations graphed in Fig. 2a–d. The results show that the pairs, SMB–BB and BF–PEI, show higher average genetic identity values than the overall values in the cross comparison between the pairs in the upper right quadrat (Table 3).

The following statistical test was used to show whether or not the pairing of localities is a chance occurrence. The identity values for each locus are assigned nonparametric values from 1 to 6. For example, the highest value, 6, is assigned to the highest identity value, while the lowest value, 1, represents the lowest identity value. The addition of any two values between 1 and 6 would result in 15 possible sums approximately normally distributed about a mean of 7 with a variance of 5. Taking all loci into account, the expected mean would be $4 \times 7/4$, or 7, and the expected variance 5/4, or 1.25. The nonparametric values assigned to identity values of SMB–BB and BF–PEI pairs and the sums of these values for each locus are presented in Table 4. The mean value, 10.5, exceeds a range of three times the standard error about the mean in a normal distribution (i.e. 7 ± 3 SE is 3.646 to 10.354). These results, therefore, indicate that there is less than a 1% chance that the apparent pairing of the genetic values of the four locations arose by chance.

Hardy–Weinberg equilibrium tests were carried out to determine whether the observed zygotic distribution conforms to what is expected under equilibrium conditions. At all loci examined, there are significant deviations (at $P = 0.05$ level) from Hardy–Weinberg equilibrium within all localities, even when the less common alleles are pooled. This appears as a deficiency in the expected proportions of heterozygotes, and is not an unusual feature of mussel populations (Levinton and Koehn 1976). The degree of heterozygote deficiency below Hardy–Weinberg expectations is expressed as the "*D*" index of Selander (1970) (Table 5). Tests for homogeneity (Sokal and Rohlf 1969) among isoenzymes and among localities show that the deficiency of heterozygotes is significantly more variable in PEI, while among isoenzymes, there is a significantly greater variation at the PGI locus.

Milkman and Beaty (1970) suggested that the apparent deficiency of heterozygotes might be explained

TABLE 2. Probability levels of paired comparisons of allele distributions using chi-square tests. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n.s. = not significant.)

LAP-1	BF	PEI	BB	SMB
BF	—	***	n.s.	**
PEI		—	***	***
BB			—	*
SMB				—

PEPTIDASE-2	BF	PEI	BB	SMB
BF	—	***	***	***
PEI		—	***	***
BB			—	n.s.
SMB				—

PGI	BF	PEI	BB	SMB
BF	—	*	***	***
PEI		—	***	***
BB			—	n.s.
SMB				—

PGM	BF	PEI	BB	SMB
BF	—	n.s.	***	***
PEI		—	***	***
BB			—	n.s.
SMB				—

by the presence of a silent or null allele. The expected frequencies for such an allele may be calculated in the following manner (E. Zouros, 1979, unpublished data):

$$(1)\quad 1 - p_s = m - \sum^{i} \sqrt{1 - a_i(1 - p_s)(1 + p_s)}$$

where p_s is the expected frequency of the silent allele at each locus; m is the number of active alleles; a_i is the sum of the observed frequencies of heterozygotes and homozygotes for each active allele. The minimum value of p_s is defined by the following equation:

$$(2)\quad p_s = 1 - \sum^{i} (1 - \sqrt{1 - a_i}).$$

Values for p_s above this minimum value are substituted into equation (1) by trial and error until the equation balances. Computation of the expected frequency of a null allele at each of the four loci at the four localities results in values ranging between 0.18 and 0.53.

TABLE 3. Among-locus average genetic identity values between pairs of localities.

	BF	PEI	BB	SMB
BF		0.951	0.882	0.835
PEI			0.804	0.753
BB				0.988
SMB				

TABLE 4. Nonparametric values assigned to identity values from Table 3 (see text) ($\bar{X} = 10.5$).

	LAP-1	PEPTIDASE-2	PGI	PGM
SMB/BB	5	6	6	6
PEI/BF	4	5	5	5
SMB/BB + PEI/BF	9	11	11	11

TABLE 5. Heterozygote deficiency indices for all localities and all loci. Probability levels (using chi-square values) resulting from tests for homogeneity among loci and localities are shown in the row and column margins, respectively. (*$P < 0.05$, ***$P < 0.001$, n.s. = not significant.)

	LAP-1	PEPTIDASE-2	PGI	PGM	*P*
BF	−0.458	−0.598	−0.396	−0.543	n.s.
PEI	−0.683	−0.559	−0.292	−0.386	***
BB	−0.539	−0.471	−0.515	−0.476	n.s.
SMB	−0.469	−0.364	−0.606	−0.514	n.s.
p	n.s.	n.s.	*	n.s.	

Discussion

The most striking feature of the results is the pairing of the allele frequency distributions among localities. The hypothesis that such a pairing exists is most strongly suggested by Fig. 2c and 2d for the PGI and PGM loci, respectively. The SMB–BB pair also shows up clearly at the Peptidase-2 locus, Fig. 2b, while the other pair, BF–PEI, appears as two distinct trend lines. The thesis of two pairs is not invalidated by Fig. 2a for the LAP-1 locus, where the two pairs show about equal separation within pairs. The near identity of BF and BB may therefore reasonably be interpreted as the result of a near overlap of the two pairs. Clearly, the pair hypothesis would not have been formulated from LAP-1 data alone. Taken as part of the general array, however, it appears reasonable to suppose that a hypothesis of paired comparisons of the samples should help to explain the observed allele distributions. This is confirmed by calculations of Table 4.

In establishing the allele frequency patterns, it is of interest that the differences among samples are the result of opposite relative frequencies at the extremes of the mobility scale, with an overall similarity of allele frequencies in the intermediate range of mobilities. This is characteristic of all cases except for the three relatively rare alleles at either end of the array for PGM. These three alleles may be an unimportant exception, and if we disregard them or pool them with the other alleles, then for all four isoenzymes the paired com-

parisons appear to arise from the fact that faster migrating alleles predominate in the BF–PEI pair. Slower migrating alleles tend to be generally more common in the SMB–BB pair than in the BF–PEI pair, and clearly predominate in the SMB–BB pair at the Peptidase-2, PGI, and PGM loci. Again, LAP-1 appears as the exception, the fast-migrating alleles predominating throughout at this locus.

This pairing suggests some kind of pairing among selective forces working in the four localities examined. This is borne out in the information we have on the aquatic environments involved. The Atlantic coast embayments exhibit a common seasonality in temperature which ranges from 2 to 20°C and 0 to 17°C in BB and SMB, respectively. Generally, salinities vary year round from only 28 to 30‰ in BB and 29.5 to 30.5‰ in SMB (Platt and Irwin 1968, 1971). However, BB has significantly more freshwater input from streams than SMB and this may lower the salinity of the surface layer in the spring. Annual production by phytoplankton is 15% higher in BB (Platt 1971) and a higher and more constant level of phytoplankton standing stock is maintained throughout the year compared with SMB (Platt 1975).

BF and PEI represent environments in which mussels are exposed to greater extremes and greater variability of a number of environmental parameters compared with the Atlantic coast embayments. PEI has greater seasonal extremes in temperature (0–25°C), as well as a wider range of salinity conditions (4–25‰) (Mr Alvin Bryan, Ellerslie Marine Station, Ellerslie, P.E.I., 1978, unpublished data). During the more severe winters, there is extensive ice cover to a maximum of 1 m thick, and usually lasting from early January to late April (Thomas 1970). The chlorophyll estimates of Uyeno (1966) from May to November are as high as those recorded in BB at about the same time (Platt and Irwin 1970), suggesting comparable primary production levels. However, this production occurs over a shorter time period, limiting organisms to a shorter growing season.

Seasonality is not as pronounced in the water in BF; year-round temperatures vary from 0 to 13°C and 27 to 31‰, respectively (C. Amos, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2, 1979 personal communication). This reduced seasonality is attributable to the extensive mixing by the tides. However, in this area, mussels were confined to the intertidal zone, and as a result of the extreme amplitude of Fundy tides, fluctuations in temperature, salinity, current flow, and food supply are considerable.

On a gradient of increasing environmental stability, the BF–PEI pair and the SMB–BB pair of environments would represent opposite ends of the scale. On this basis the pairing of the allele frequency distributions suggests a strong parallelism of selective forces within pairs. It is striking, but perhaps coincidental, that this should be reflected by generally more mobile alleles in the more variable environment, leading to the inference that this difference might somehow reflect a functional aspect of their adaptability.

It is clear from our results that whatever selective forces are involved, they affect the various loci differently. For example, the differences between the pairs of localities showed up most strongly in the Peptidase-2 and PGI loci (Fig. 2b and 2c). Within pairs, BF and PEI showed significant differences at all except the PGM locus. By contrast, the SMB–BB pair showed a significant difference at the LAP-1 locus only. Assuming independent assortment at the four loci, it may be concluded that there is a different sensitivity of the enzyme systems to environmental selective forces.

In this connection, the implication of the LAP-1 locus in adaptation to salinity (Koehn et al. 1976) is consistent with the results in Table 2. For example, the BB–BF value indicates similarity of genetic constitution at this locus. BB is subject to occasional low salinities at the surface, a feature it shares with intertidal BF, and in which it differs from SMB. PEI and SMB represent opposite ends of the scale in terms of stability of salinity conditions. This may be the reason for the especially large genetic difference between PEI and SMB at this locus, as well as the small but statistically significant difference between SMB and BB. Although the underlying selective mechanism for maintaining this polymorphism and its adaptive significance is still poorly understood, Koehn 1978 has elucidated some functional biochemical aspects of LAP-1. He has shown that the activity of LAP-1 is correlated with environmental salinity, and has demonstrated differences in activity among natural mussel populations from marine and estuarine environments.

While it is apparent that environmental influences may be affecting our results, it must be recognized that gene flow between localities may be a contributing factor. This is true in the case of neighboring SMB and BB which are subject to periodic tidal and wind-driven flushing and considerable longshore water movement (Platt et al. 1972) which could mix the populations during the presettlement stage. There is also a recognizable net water movement from the PEI area towards the SMB–BB area. However, the genetic differences between the two pairs of areas were large, suggesting that gene flow from the PEI area is minimal. The genetic similarity of BF and PEI must in fact be a consequence of similar environments since larvae dispersing between these two areas would have to bypass SMB and BB. This conclusion also implies that gene flow effects can be invoked to account only for the smaller difference within the SMB–BB pair than within the BF–PEI pair, and is not necessarily sufficient to account for the fact that the SMB–BB mussels showed similar gene frequencies at three loci.

It is of some practical importance to compare this result for gene frequency distributions between SMB and BB with results of Freeman and Dickie (1979) comparative production observations. They compared growth and mortality of mussels from a common source

stock held for 18 mo in these two bays. Their results indicating significant production differences between the areas, when compared with ours, suggest that selection effects on the four loci are not strong enough to allow us to use gene frequency distributions to index short-term environmental differences. It would seem desirable to carry out further experiments to determine on what scales the selection-mediated environmental gradient effects are overbalanced by gene flow.

In all four localities we observed a large deficiency of heterozygotes at each locus. The hypothesis that this might be explicable in terms of silent alleles was examined by the calculation of equations 1 and 2. For the hypothesis to work, it must be assumed that the homozygote is lethal and that the silent alleles occur in the heterozygous form only, making our identification of excessive homozygotes faulty. The frequencies of silent alleles required are very high (0.18 to 0.53 depending on locus and locality) and nonlethal heterozygotes could only be maintained at such levels if there were very high mutation rates at all four loci, or if silent alleles were highly overdominant for fitness (E. Zouros et al., 1979, unpublished data). Neither possibility is strong enough for silent alleles to provide a convincing explanation for the high heterozygote deficiencies found at all four loci and localities.

An excess of homozygotes in a population could possibly result from inbreeding in various stocks or from the Wahlund effect, which is the settling in the sample areas of individuals from effectively very small parent populations within the bays and which over time had developed significantly different genetic constitutions.

The amount of inbreeding needed to produce deficiencies in heterozygosities of the observed magnitude may be calculated as the negative grand mean of the *D* values of Table 5 and has a value of 0.49. Inbreeding of this magnitude is highly unlikely in view of reproduction by external fertilization and the relatively long pelagic larval stage which should permit rather thorough mixing of progeny. In addition, for the Wahlund effect to provide an explanation, we would expect that all loci would be equally affected within a given locality. In at least one locality, PEI, it can be seen that this is not so (Table 5). We, therefore, are led to conclude that selection due to environmental factors at a microgeographical scale, either alone or in conjunction with these other "stock dynamics" effects, provides the most likely explanation for the heterozygote deficiency in these mussel populations. Koehn et al. (1976) have reached a similar conclusion for the mussel populations they examined.

While we know very little about the specific functions associated with isoenzymes, the evidence that environmental factors are associated with the genetic differences suggests that there must have been adaptation of the physiological and production responses among stocks. Such adaptation would be expected to optimize aspects of the production system, and may have altered the physiological responses of stocks to given environmental variables. The possible variations in response would have to be taken into account in any attempt to use the mussel as an environmental index organism.

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Population or Stock Structure of Lake Whitefish, *Coregonus clupeaformis*, in Northern Lake Michigan as Assessed by Isozyme Electrophoresis

MARK IMHOF,[1] ROBB LEARY, AND HENRY E. BOOKE

Wisconsin Cooperative Fishery Research Unit, Stevens Point, WI, USA[2]

IMHOF, M., R. LEARY, AND H. E. BOOKE. 1980. Population or stock structure of lake whitefish, *Coregonus clupeaformis*, in northern Lake Michigan as assessed by isozyme electrophoresis. Can. J. Fish. Aquat. Sci. 37: 783–793.

Electrophoretic analysis of three polymorphic isozymes (lactate, malate, and glycerol-3-phosphate dehydrogenases) from lake whitefish, *Coregonus clupeaformis*, indicated that at least four populations exist in northern Lake Michigan. Cluster analysis of Nei genetic identity indices revealed much temporal and spatial overlap among population ranges. The overlap did not appear complete since the LDH *A* allele exhibited clinal variation with increasing frequency from south to north. Because population ranges overlap spatially and temporally, commercial catches are likely to contain fish from more than one population.

Key words: population (stock), isozyme electrophoresis, lake whitefish, Lake Michigan

IMHOF, M., R. LEARY, AND H. E. BOOKE. 1980. Population or stock structure of lake whitefish, *Coregonus clupeaformis*, in northern Lake Michigan as assessed by isozyme electrophoresis. Can. J. Fish. Aquat. Sci. 37: 783–793.

Une analyse électrophorétique de trois isozymes polymorphes (lactate, malate et glycérol-3-phosphate déshydrogénases) de grande corégone, *Coregonus clupeaformis*, revèle la présence d'au moins quatre populations dans la partie septentrionale du lac Michigan. L'analyse des grappes d'indices d'identité génétique démontre qu'il y a chevauchement temporel et spatial prononcé de l'aire de ces populations. Le chevauchement ne semble toutefois pas complet, puisque l'allèle A de la LDH accuse une variation clinale, la fréquence augmentant du sud au nord. Comme l'aire de ces populations chevauche dans l'espace et dans le temps, les prises commerciales contiendront probablement des poissons de plus d'une population.

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LAKE whitefish (*Coregonus clupeaformis*) in Lake Michigan has supported a commercial fishery since the mid-1800s (Baldwin and Saalfeld 1962; Smith 1968). Notwithstanding its importance as a commercial species, little information is available on the number of populations of lake whitefish in Lake Michigan. The identification of populations (= stocks) and their temporal and spatial ranges will be valuable information for efficient management and preservation of the commercial fishery (Fukada 1973; Larkin 1977).

A population is ideally defined as a group of intraspecific individuals so situated that any two of them have an equal probability of mating, provided that they are mature and of the opposite sex, i.e. breeding is a random process (Mayr 1963). All members of a population form a common gene pool, and it is within this pool that genes interact among themselves and the environment in various combinations, genotypes. The continual interaction of genes in the pool produces a degree of genetic integration such that, evolutionarily, the population is a single unit and, therefore, all samples from a population should have the same allele frequencies (with sampling error) at a particular locus.

Vertical starch gel electrophoretic analysis of three polymorphic isozyme systems (lactate, malate, and glycerol-3-phosphate dehydrogenases, all NAD^+ dependent) was used in an attempt to identify the number of populations of lake whitefish and their temporal and spatial ranges in northern Lake Michigan (statistical districts MM1–MM4, WM1–WM5; Smith et al. 1961). The population genetics approach was selected because of the extreme morphological plasticity present within coregonine fish (Koelz 1929; Svärdson 1970; Loch 1974).

Methods

Beginning with the 1975 fall spawning season and end-

[1]The first two authors have contributed equally and are alphabetically listed. M. Imhof's present address is the University of Wisconsin, Laboratory of Limnology, Madison, WI 53706.

[2]U.S. Fish and Wildlife Service, Wisconsin Department of Natural Resources and the University of Wisconsin–Stevens Point cooperating.

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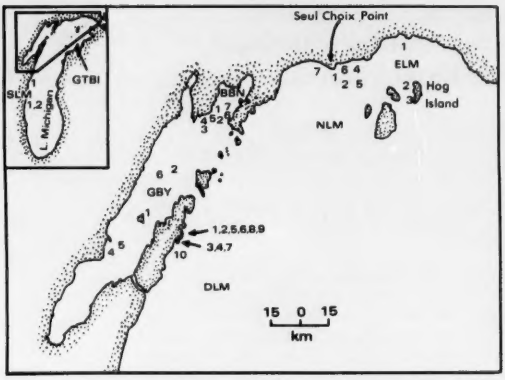

FIG. 1. Lake whitefish sample sites in northern Lake Michigan. SLM = southern Lake Michigan, DLM = Door County, GBY = Green Bay, BBN = Big Bay de Noc, NLM = northern Lake Michigan, ELM = eastern Lake Michigan, GTB = Grand Traverse Bay. Numbers represent the locations of individual samples and correspond with the sample numbers in Table 1.

ing with the 1978 spawning season, 2295 lake whitefish were obtained from commercial fishermen, in 35 samples, from northern Lake Michigan (Fig. 1; for date of collection, gear, and depth of each sample, see Leary 1979). Selection of sampling sites was based on the location of commercial operations. Samples were collected in spring, summer, and fall to investigate the possibility that gene frequencies changed seasonally. Fall samples were limited to areas that appeared to be in the vicinity of lake whitefish spawning grounds because the commercial catches contained ripe fish.

Fish were put on ice after purchase and transported to a laboratory; total length of some individuals was recorded to the nearest millimetre, and the fish were frozen at −20°C. When red and white muscle tissues were required for electrophoresis, the fish were partially thawed, scales removed for aging, and 2 g of red and white muscle was excised from the area between the lateral line and the dorsal fin. Tissues were cooled in an ice bath and simultaneously homogenized in a 15-mL Pyrex glass tissue grinder in 5 mL of 0.0001 mol/L beta-nicotinamide adenine dinucleotide (NAD^+). Homogenates did not require centrifugation for resolution of isozyme bands. The homogenates were poured into 2-mL sealable plastic test tubes and stored at −70°C for subsequent electrophoresis.

Lactate (LDH), malate (MDH), and glycerol-3-phosphate (G-3-PDH) dehydrogenase isozymes were separated by vertical starch gel electrophoresis with 20–25 mA/gel in an environmental chamber at 5°C. Electrostarch, stains, and buffers were prepared according to the methods of Clayton and Tretiak (1972). After 16 h, electrophoresis was terminated, and the gels stained for the desired isozymes.

The band patterns on the electropherograms were scored according to genetic models (Clayton and Franzin 1970, Clayton et al. 1973) which in the absence of breeding data were accepted as correct if the following two criteria of Utter et al. (1974) were met:

1) Band patterns must be repeatable with subsampling of the same tissue of an organism.
2) The bands resolved must fit interpretable patterns based on genetic hypotheses.

LDH and MDH red muscle isozymes were scored, and for white muscle G-3-PDH, β locus isozymes were scored.

If only one population was sampled, the frequency of alleles at a locus in each sample would be expected to be the same with sampling error, i.e. the allele frequencies should form a homogenous group. Three contingency tables were constructed using the isozyme data from the various samples. Homogeneity within the contingency tables was analyzed by chi-square tests.

A Nei normalized genetic identity index (Nei 1972, 1975) was calculated between each sample with all other samples. Unweighted pair group hierarchal cluster analysis (Sneath and Sokal 1974) was used on a matrix of the Nei identity indices to form genetically similar groups of samples. If contingency tables revealed heterogeneity, inspection of sample location and collection dates within the groups formed by the cluster analysis could offer some indication of the temporal and spatial ranges of the populations in the study area. The computer program of McCammon and Wenniger (1970) was used for the clustering.

Association between latitude or longitude and allele frequencies was tested by linear regression analysis to investigate the possibility that gene frequencies might exhibit clinal variation.

To estimate comparative amounts of heterozygosity within year-classes, we calculated a direct measure of heterozygosity adjusted for gene frequency from the observed $\Sigma_{ij}P_iP_j$ divided by the expected $\Sigma_{ij}P_iP_j$ (J. Crow, personal communication) for the 1972 through 1975 year-classes within a cluster, where P_iP_j represents a heterozygous individual. The above equation is a ratio of how well the sum of the observed number of heterozygotes conforms with the sum of the expected number based on Castle–Hardy–Weinberg equilibrium. Values greater than one indicate an excess of observed heterozygotes, and values less than one, a deficit.

Results and Discussion

Three, five, and six phenotypes (= band patterns) were observed on the electropherograms for LDH, MDH, and G-3-PDH, respectively. All phenotypes were identical with those observed by Clayton and Franzin (1970), Lindsey et al. (1970), and Clayton et al. (1973), and were scored, with the exception of MDH, according to their models. As Franzin and Clayton (1977) reported, the MDH phenotype was sometimes found to differ between red and white muscle homogenates from the same individual. A mixture of red and white muscle homogenates, however, was found always to express the red phenotype. It was this phenotype that was scored from the electropherograms with the genetic model proposed in Fig. 2. Two loci, each with two alleles, are postulated. Because the slower, anodally migrating alleles at each locus have the same mobility, and the fast migrating alleles have the same mobility, only one or three bands are produced on the electropherograms by the isozymes produced by these loci.

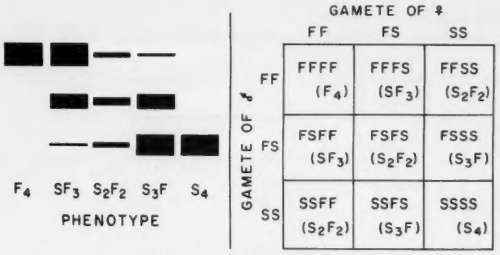

FIG. 2. MDH lake whitefish electrophoretic phenotypes for the two most anodally migrating loci. Punnett square depicts gamete combinations and the resultant genotypes (upper) and phenotypes (lower).

Equal mobility of the MDH isozymes precluded the determination of allele frequencies. For example, if locus one were SS and locus two FF, the result would be an S_2F_2 phenotype (Fig. 2); however, the same phenotype would be produced if both loci were SF. The S_3F and SF_3 phenotypes could also be produced by different genotypes. Since allele frequencies for the MDH loci were not calculable, the frequencies of the MDH gametes (SS, SF, FF) were estimated from the observed phenotypic distributions with a maximum likelihood method (J. Crow personal communication) assuming disomic inheritance, and the two loci are not linked (Appendix).

Evidence for Genetic Heterogeneity Among the Samples

The contingency table chi-square analyses of the LDH and G-3-PDH allele distributions (Tables 1 and 2, respectively) and the MDH gametes (Table 3) showed significant departures from homogeneity for the LDH alleles (H_o = allele distributions in each sample are the same; $P < 0.05$) and MDH gametes (H_o = gamete distributions in each sample are the same $P < 0.001$). The G-3-PDH allele distributions could reasonably be considered homogenous throughout the study area (H_o = allele distributions in each sample are the same; $P > 0.25$). Because the estimation of MDH gametes by maximum likelihood produces larger error envelopes around the estimates than those encountered by counting alleles, the χ^2 results from this system may be suspect. However, the highly significant χ^2 value and that it is not the only system that indicates heterogeneity support the hypothesis that not all the heterogeneity among the MDH gametes is an artifact of the estimation procedure.

In the LDH data there was an area of persisting monomorphism (frequency of the commonest allele >0.990) in the vicinity of Hog Island, Michigan (ELM2, 3; Fig. 1 and Table 1), and extending at times as far west as Seul Choix Point, Michigan (NLM4, 5; Fig. 1 and Table 1). The claim that the monomorphic samples are due to error introduced by finite sampling

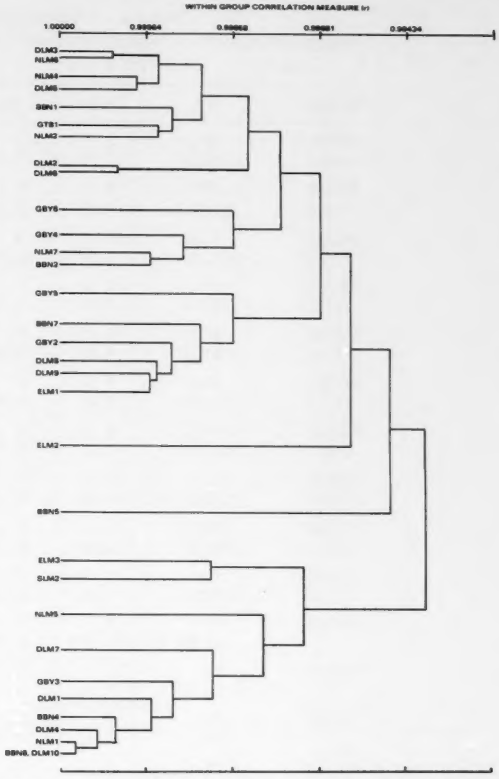

FIG. 3. Cluster analysis of Nei similarity indices from lake whitefish sample comparisons.

would be tenuous because the monomorphic samples were restricted only to the northeastern sector of the study area, not randomly throughout, and of its persistence in the vicinity of Hog Island. It may be worthy to note that if the four monomorphic samples are excluded from the LDH contingency table the χ^2 test no longer indicates heterogeneity.

The significant departures from homogeneity indicated by the χ^2 analyses and the existence of polymorphic and monomorphic samples at the same locus are not compatible with the hypothesis that only one population was sampled. These results suggest that two or more populations of lake whitefish exist in northern Lake Michigan.

Evidence for Temporal and Spatial Overlap Among Population Ranges

The dendrograph produced by clustering the Nei identity indices (range: 0.9997–0.9033) revealed two main clusters (Cluster I = DLM3–ELM1; Cluster II = ELM3–DLM10; Fig. 3) with two outlying samples

TABLE 1. LDH red muscle isozyme frequencies.

Sample	Phenotypes			Sample size	Alleles	
	AA	AB	BB		*A*	*B*
BBN1	48	2	0[a]	50	98	2[b]
	48.01	1.95	0.01		0.979	0.019
NLM1	57	4	0	61	118	4
	57.06	3.86	0.06		0.967	0.032
DLM1	54	2	2	58	110	6
	52.15	5.68	0.15		0.948	0.051
DLM2	58	2	0	60	118	2
	58.01	1.96	0.01		0.983	0.016
GBY1	44	4	0	48	92	4
	44.08	3.83	0.08		0.958	0.041
PID1	22	1	0	23	45	1
	22.01	0.97	0.01		0.978	0.021
SLM1	26	4	0	30	56	4
	26.13	3.73	0.13		0.933	0.066
BBN2	63	5	1	69	131	7
	62.17	6.64	0.17		0.949	0.050
NLM2	64	9	0	73	137	9
	64.27	8.44	0.27		0.938	0.061
ELM1	58	2	0	60	118	2
	58.01	1.96	0.01		0.983	0.016
DLM3	55	4	0	59	114	4
	55.06	3.86	0.06		0.966	0.033
GBY2	53	5	2	60	111	9
	51.33	8.32	0.33		0.924	0.074
GBY3	58	2	0	60	118	2
	58.01	1.96	0.01		0.983	0.016
NLM4	77	1	0	78	155	1
	77.00	0.99	0.00		0.993	0.006
BBN4	55	2	0	57	112	2
	55.01	1.96	0.01		0.982	0.017
DLM4	77	6	0	83	160	6
	77.10	5.78	0.10		0.963	0.036
GBY4	65	7	0	72	137	7
	65.17	6.65	0.17		0.951	0.048
DLM5	132	12	0	144	276	12
	132.24	11.49	0.24		0.958	0.041
BBN5	52	8	0	60	112	8
	52.26	7.46	0.26		0.933	0.006
NLM5	65	0	0	65	130	0
	65.00	0.00	0.00		1.000	0.000
ELM2	46	0	0	46	92	0
	46.00	0.00	0.00		1.000	0.000
DLM6	53	6	0	59	112	6
	53.15	5.69	0.15		0.949	0.050
DLM7	75	7	0	82	157	7
	75.14	6.70	0.14		0.957	0.042
DLM8	45	4	0	49	94	4
	45.08	3.83	0.08		0.959	0.040
GTB1	59	5	0	64	123	5
	59.09	4.80	0.09		0.960	0.039

TABLE 1 (*Concluded*)

Sample	Phenotypes			Sample size	Alleles	
	AA	AB	BB		*A*	*B*
GBY5	89	11	1	100	189	13
	88.41	12.16	0.41		0.935	0.064
DLM9	58	4	1	63	120	6
	57.14	5.71	0.14		0.952	0.047
SLM2	70	8	0	78	148	8
	70.20	7.58	0.20		0.948	0.051
DLM10	83	10	0	93	176	10
	83.26	9.46	0.26		0.946	0.053
BBN6	70	5	0	75	145	5
	70.08	4.83	0.08		0.966	0.033
NLM6	62	6	0	68	130	6
	62.13	5.73	0.13		0.955	0.044
NLM7	60	6	0	66	126	6
	60.13	5.72	0.13		0.954	0.045
BBN7	43	5	1	49	91	7
	42.24	6.49	0.24		0.923	0.071
ELM3	65	0	0	65	130	0
	65.00	0.00	0.00		1.000	0.000
GBY6	67	3	0	70	137	3
	67.03	2.93	0.03		0.978	0.021

[a]Observed number of phenotypes is printed above the expected number.
[b]Allele frequencies are tabulated below the number of alleles.

TABLE 2. G-3-PDH β locus isozyme frequencies.

Sample	Phenotypes						Sample size	Alleles		
	B1B1	B1B2	B1B3	B2B2	B2B3	B3B3		*B1*	*B2*	*B3*
BBN1	3	16	4	14	12	1[a]	50	26	56	18[b]
	3.37	14.55	4.67	15.67	10.07	1.61		0.259	0.559	0.179
NLM1	7	20	5	20	8	1	61	39	68	15
	6.23	21.73	4.79	18.95	8.36	0.92		0.319	0.557	0.122
DLM1	7	15	6	18	11	1	58	35	62	19
	5.28	18.70	5.73	16.56	10.15	1.55		0.301	0.534	0.163
DLM2	5	21	5	19	8	2	60	36	67	17
	5.39	20.09	5.09	18.70	9.49	1.20		0.299	0.558	0.141
GBY1	6	14	5	15	6	2	48	31	50	15
	5.00	16.14	4.84	13.02	7.81	1.17		0.322	0.520	0.156
PID1	2	6	3	6	5	1	23	13	23	10
	1.83	6.49	2.82	5.75	4.99	1.08		0.282	0.500	0.217
SLM1	4	8	6	6	5	1	30	22	25	13
	4.03	9.16	4.76	5.20	5.41	1.40		0.366	0.416	0.216
BBN2	7	29	4	18	8	3	69	47	73	18
	8.00	24.86	6.13	19.30	9.52	1.17		0.340	0.528	0.130
NLM2	7	19	7	24	12	4	73	40	79	27
	5.47	21.64	7.39	21.37	14.60	2.49		0.273	0.541	0.184
ELM1	8	24	5	14	9	0	60	45	61	14
	8.43	22.87	5.24	15.50	7.11	0.81		0.375	0.508	0.116
DLM3	4	19	6	24	5	1	59	33	72	13
	4.61	20.13	3.63	21.96	7.93	0.71		0.279	0.610	0.110

TABLE 2 (*Concluded*)

Sample	Phenotypes						Sample size	Alleles		
	B1B1	B1B2	B1B3	B2B2	B2B3	B3B3		*B1*	*B2*	*B3*
GBY2	8	23	5	17	7	0	60	44	64	12
	8.06	23.46	4.39	17.06	6.39	0.59		0.366	0.533	0.099
GBY3	7	15	2	16	19	1	60	31	66	23
	4.00	17.04	5.94	18.14	12.64	2.20		0.258	0.549	0.191
NLM4	7	25	4	27	13	2	78	43	92	21
	5.92	25.35	5.78	27.12	12.38	1.41		0.275	0.589	0.134
BBN4	8	18	4	17	9	1	57	38	61	15
	6.33	20.33	4.99	16.32	8.02	0.98		0.333	0.535	0.131
DLM4	5	33	6	24	12	3	83	49	93	24
	7.23	27.45	7.08	26.05	13.44	1.73		0.295	0.560	0.144
GBY4	6	22	10	20	11	3	72	44	73	27
	6.72	22.30	8.24	18.50	13.68	2.53		0.305	0.506	0.187
DLM5	6	34	6	26	17	2	91	52	103	27
	7.42	29.42	7.71	29.14	15.28	2.00		0.285	0.565	0.148
BBN5	4	29	7	12	7	1	60	44	60	16
	8.06	21.99	5.86	15.00	7.99	1.06		0.366	0.500	0.133
NLM5	4	23	7	20	10	1	65	38	73	19
	5.55	21.33	5.55	20.49	10.66	1.38		0.292	0.561	0.146
ELM2	7	18	4	7	8	2	46	36	40	16
	7.04	15.65	6.26	8.69	6.95	1.39		0.391	0.434	0.173
DLM6	8	16	4	20	11	0	59	36	67	15
	5.49	20.44	4.57	19.02	8.51	0.95		0.305	0.567	0.127
DLM7	6	26	14	15	18	3	82	52	74	38
	8.24	23.46	12.04	16.69	17.14	4.40		0.317	0.451	0.231
DLM8	5	17	6	14	6	1	49	33	51	14
	5.55	17.17	4.71	13.27	7.28	0.99		0.336	0.520	0.142
GTB1	9	16	5	16	17	1	64	39	65	24
	5.94	19.80	7.31	16.50	12.18	2.25		0.304	0.507	0.187
GBY5	20	33	12	21	11	3	100	85	82	29
	18.06	36.54	12.32	18.48	12.46	2.10		0.424	0.429	0.144
DLM9	9	24	6	16	7	1	63	48	63	15
	9.14	23.99	5.71	15.75	7.49	0.89		0.380	0.500	0.119
SLM2	11	23	11	20	10	3	78	56	73	27
	10.05	26.20	9.69	17.08	12.63	2.33		0.358	0.467	0.173
DLM10	10	31	6	31	14	1	93	57	107	22
	8.73	32.79	6.74	30.77	12.65	1.30		0.306	0.575	0.118
BBN6	8	25	5	26	8	2	74	46	85	17
	7.14	26.41	5.28	24.40	9.76	0.97		0.310	0.574	0.114
NLM6	6	19	8	26	9	0	68	39	80	17
	5.59	22.94	4.87	23.52	9.99	1.06		0.286	0.588	0.125
NLM7	5	24	7	18	10	2	66	41	70	21
	6.36	21.74	6.52	18.56	11.13	1.67		0.310	0.530	0.159
BBN7	5	23	3	11	7	1	50	36	52	12
	6.47	18.71	4.31	13.51	6.23	0.71		0.359	0.519	0.119
ELM3	9	21	5	13	12	5	65	44	59	27
	7.44	19.96	9.13	13.38	12.25	2.80		0.338	0.453	0.207
GBY6	5	21	6	23	11	4	70	37	78	25
	4.88	20.61	6.60	21.72	13.92	2.23		0.264	0.557	0.178

[a]Observed number of phenotypes is printed above the expected number.
[b]Allele frequencies are tabulated below the number of alleles.

TABLE 3. MDH red muscle isozyme gamete frequencies.

Sample	Phenotypes					Sample size	Gametes		
	S4	S3F	S2F2	SF3	F4		SS	SF	FF
BBN1	1	4	14	20	11[a]	50	11.5	40.9	47.5[b]
	0.66	4.72	13.85	19.44	11.30		0.115	0.409	0.475
NLM1	0	3	19	14	12	48	6.9	47.0	41.9
	0.25	3.41	14.58	20.57	9.17		0.072	0.490	0.437
DLM1	0	4	18	25	8	55	6.3	60.2	43.3
	0.18	3.49	19.00	23.75	8.55		0.058	0.547	0.394
DLM2	1	9	19	17	13	59	21.6	42.7	53.6
	1.98	7.83	17.57	19.42	12.18		0.183	0.362	0.454
BBN2	1	7	16	27	18	69	16.6	50.7	70.6
	1.00	6.11	17.83	25.96	18.07		0.120	0.367	0.511
NLM2	0	7	18	31	17	73	12.5	62.8	70.5
	0.54	5.41	19.60	30.37	17.06		0.068	0.430	0.483
ELM1	0	6	20	19	15	60	13.9	49.1	56.9
	0.80	5.70	16.67	23.31	13.50		0.116	0.409	0.474
DLM3	0	6	18	22	13	59	11.4	53.1	53.4
	0.55	5.15	17.13	24.05	12.10		0.096	0.450	0.452
GBY2	1	8	15	24	12	60	16.3	49.3	54.4
	1.10	6.71	17.54	22.34	12.29		0.135	0.411	0.452
GBY3	0	4	23	19	14	60	8.4	60.0	51.4
	0.30	4.24	18.64	25.75	11.04		0.070	0.500	0.429
NLM4	1	2	25	29	21	78	10.5	67.9	77.5
	0.35	4.58	20.02	33.75	19.27		0.067	0.435	0.497
BBN4	0	3	20	21	13	57	6.0	57.8	50.0
	0.16	3.08	17.34	25.40	10.99		0.053	0.507	0.439
DLM4	0	7	26	35	15	83	11.9	84.0	69.9
	0.43	6.07	26.31	35.42	14.75		0.072	0.506	0.421
GBY4	1	7	23	23	18	72	21.3	51.3	71.3
	1.57	7.60	19.71	25.44	17.65		0.147	0.356	0.495
DLM5	0	6	21	39	24	90	11.9	75.1	92.9
	0.39	4.97	21.84	38.80	23.97		0.066	0.417	0.516
BBN5	0	5	31	10	14	60	33.3	20.2	66.3
	4.64	5.63	20.16	11.20	18.35		0.278	0.168	0.553
NLM5	0	1	29	26	9	65	1.5	83.8	44.5
	0.00	1.00	27.59	28.75	7.63		0.012	0.645	0.342
ELM2	0	3	25	10	8	46	22.9	23.0	45.9
	2.87	5.75	14.37	11.50	11.49		0.249	0.250	0.499
DLM6	2	8	18	19	12	59	22.1	42.7	53.1
	2.07	8.01	17.70	19.24	11.96		0.187	0.362	0.450
DLM7	1	6	26	33	16	82	14.8	77.3	71.8
	0.67	6.99	24.73	33.87	15.72		0.090	0.471	0.437
DLM8	1	5	15	18	10	49	13.2	40.4	44.2
	0.89	5.47	14.34	18.28	9.99		0.135	0.413	0.451
GTB1	1	6	17	27	13	64	14.0	54.9	59.0
	0.76	6.01	18.26	25.34	13.60		0.109	0.429	0.461
GBY5	2	10	34	34	20	100	27.9	84.1	87.9
	1.94	11.74	29.98	36.99	19.32		0.139	0.420	0.439
DLM9	0	6	24	18	15	63	14.0	56.0	55.9
	0.77	6.22	18.66	24.88	12.44		0.111	0.444	0.444
SLM2	1	10	33	23	11	78	19.2	84.4	52.2
	1.18	10.43	29.32	28.30	8.75		0.123	0.541	0.335

TABLE 3 (*Concluded*)

Sample	Phenotypes					Sample size	Gametes		
	S4	S3F	S2F2	SF3	F4		SS	SF	FF
DLM10	0	6	38	25	24	93	15.0	88.9	82.0
	0.60	7.18	27.89	39.22	18.08		0.080	0.478	0.441
BBN6	1	4	23	31	15	74	11.4	70.1	66.4
	0.44	5.41	21.75	31.48	14.90		0.077	0.474	0.448
NLM6	2	2	22	27	15	68	13.5	57.9	64.5
	0.67	5.76	18.76	27.49	15.30		0.099	0.426	0.474
NLM7	0	6	14	26	20	66	12.0	47.9	72.0
	0.54	4.36	15.27	26.18	19.63		0.090	0.363	0.545
BBN7	0	9	15	18	8	50	13.9	47.1	38.9
	0.96	6.56	16.54	18.35	7.56		0.139	0.471	0.389
ELM3	0	8	23	25	9	65	12.4	70.0	47.4
	0.59	6.71	23.43	25.58	8.66		0.095	0.538	0.365
GBY6	1	4	20	23	22	70	18.5	41.9	79.5
	1.22	5.55	16.80	23.84	22.57		0.132	0.299	0.567

[a]Observed number of phenotypes is printed above the expected number.
[b]Gamete frequencies are tabulated below the number of gametes.

(BBN5, ELM2). In computing the Nei identity indices only the polymorphic isozyme systems were used, and the MDH gamete frequencies were treated as allele frequencies from a single locus.

The spatial and temporal overlap between the clusters was extensive and was interpreted to indicate overlap of population ranges in both time and space. Both clusters contained samples collected at the beginning of the fall spawning season in Door County, Wisconsin, and Big Bay de Noc and Seul Choix Point, Michigan. Thus population ranges apparently overlap even during the beginning of the spawning season. Therefore, mechanisms or factors other than geographical separation of spawning grounds must be operating to prevent sufficient gene flow throughout the study area that would produce the genetic integration of all individuals into one gene pool.

The possibility that populations were using the same spawning grounds but spawning at different times or depths was investigated in the fall of 1977. Beginning October 14 and ending November 11, three samples were collected, at 2-wk intervals, from North and Moonlight Bays, Door County, Wisconsin (DLM6, 7, 8; Fig. 1). The first and last of these samples (DLM6, 8) fell within one cluster and the middle sample in the other cluster (Fig. 3). These results do not support the hypothesis that one population begins spawning only after another has completed or nearly completed spawning. Samples DLM6 and 7 were caught in 10- to 17-m depth, and DLM8 from 20 to 30 m; hence the data also do not suggest habitat separation by depth as a reproductive isolating mechanism.

Habitat separation by depth or time cannot be conclusively eliminated as possible reproductive isolating mechanisms on the presumed Door County spawning grounds because the three samples were not composed of all ripe individuals and none was positively obtained directly on spawning grounds. The location of spawning grounds are hypothesized from the capture of ripe fish in fall and of larvae in spring. The area fished by a commercial net is extensive, and there is no a priori reason to conclude that the capture of ripe individuals indicates the location of a spawning ground (although it reasonably can be assumed to be close by). The presence of older larvae may also not indicate the location of spawning grounds because whitefish larvae are subject to dispersal by water currents (Hoagman 1973).

Other possible isolating mechanisms could involve specific within area homing and behavioral differences between populations. If populations respond differently to temperature, then wind-generated currents and seiches (Sato and Mortimer 1975) may influence spawning location and time. Many commercial fishermen believe that lake whitefish behavior is strongly influenced by currents. Behavioral differences allowing for discreteness in other coregonine populations have been reported by Fenderson (1964), Svärdson (1965), Lindström (1970), and Lindsey et al. (1970).

One difference between the geographic ranges of the clusters during the non-spawning season (spring and summer) was apparent. All samples from Green Bay south of Washington Island were contained only in Cluster I. The interpretation of these results to suggest that fish in southern Green Bay during spring and summer come from only one population would be tenuous. The samples were obtained from commercial catches and those nets are usually emptied at 2- to 3-day intervals. The data indicates much temporal and spatial overlap, and this, coupled with the duration of a commercial net set, makes it extremely likely that a commercial catch often contains a mixture of populations. Thus many of the samples probably contain fish from more than one population. The uniform cluster-

ing of the southern Green Bay samples may be due to a more constant proportion of mixing among populations in commercial catches as compared to a highly variable degree of mixing in the other areas sampled.

Evidence that Population Range Overlap is Not Complete

Although the samples collected at and east of Seul Choix Point fell within both clusters, the following evidence suggests that fish east of Seul Choix Point and those from Door County and Big Bay de Noc do not mix freely. A total of 8400 lake whitefish were tagged in North and Moonlight Bays, Door County, at the beginning of the fall spawning seasons of 1975 through 1978 (M. Ebener, Wisconsin Cooperative Fishery Research Unit, unpublished data). At the beginning of the 1977 and 1978 spawning seasons, 1780 fish were tagged in Big Bay de Noc (M. Ebener unpublished data). Recovered tags were centered from Sheboygan, Wisconsin, northwards to Big Bay de Noc including Green Bay. Less than 0.5% of the recovered tags came from east of Seul Choix Point, and none were recovered from Grand Traverse Bay, Michigan.

Results of linear regression analysis indicated a significant positive regression between the LDHα *A* allele and latitude (H_o = variation in allele frequency is not related to variation in latitude; $P < 0.05$). Neither the G-3-PDH alleles nor the MDH gametes exhibited latitudinal or longitudinal clinal variation. The LDH alleles did not exhibit longitudinal clinal variation.

The paucity of tag returns east of Seul Choix Point and the clinal variation of the LDHα *A* allele support the hypothesis that although population ranges overlap, the overlap is not complete.

Other Evidence of Multiple Populations of Lake Whitefish in Northern Lake Michigan

Other authors have found evidence of more than one population of lake whitefish in northern Lake Michigan. Caraway (1951) found age structure, growth, and morphometric differences between whitefish from Big Bay de Noc and north central Lake Michigan (High and Gull Islands), but no differences in the above characters between Big Bay de Noc and St. Helena Island samples (north eastern Lake Michigan). The tag returns of Roelofs (1958) showed no movement of fish from Big Bay de Noc to St. Helena Island. Piehler (1967) found that year-class strength differed between Big Bay de Noc and Hog Island samples. Mraz (1967) and Gunderson (1978) found age structure differences between samples collected in Green Bay and Lake Michigan west of Seul Choix Point.

Speculation for a Minimum of Four Populations of Lake Whitefish in Northern Lake Michigan

Considering all the data collectively a minimum of four populations of lake whitefish may be hypothesized, at least two centered to the west of Seul Choix Point and at least two to the east. The contingency table χ^2 analyses and the existence of polymorphic and monomorphic samples in the LDH data suggest that more than one population was sampled. Based upon the dispersal pattern of tag returns, that is probably greater than the extent of successful gene flow (Endler 1977), it appears that whitefish east of Seul Choix Point do not freely interbreed with those in Door County and Big Bay de Noc. The hypothesis of only one population of lake whitefish east of Seul Choix Point is not consistent with the presence of polymorphic and monomorphic samples in the LDH data. Heterogeneity among the MDH gametes from Door County, Big Bay de Noc, and Green Bay samples and the existence of a mid-winter spawning population in Green Bay (Leary 1979) suggest that a minimum of two populations are centered to the west of Seul Choix Point.

Whether the lake whitefish in Lake Michigan form good reproductively isolated populations with little gene flow among them, or form poorly isolated populations with gene flow bringing about some degree of genetic exchange, albeit not sufficient to integrate all lake whitefish into one gene pool, cannot be determined from the data. The dispersal pattern of tag returns and the

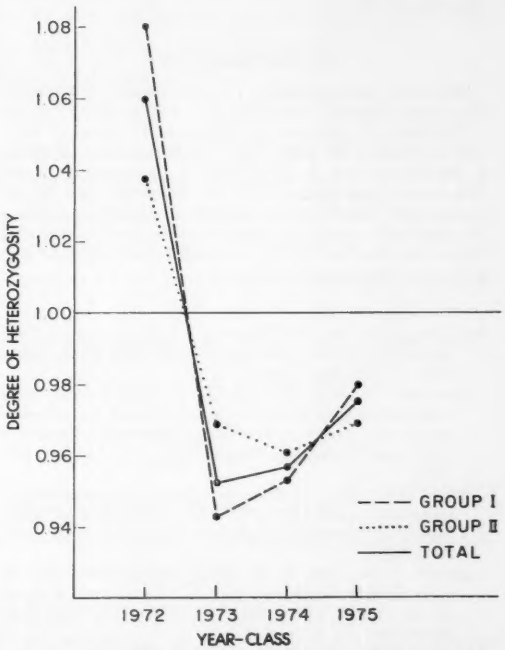

FIG. 4. Graphic representation of the degree of heterozygosity independent of gene frequency for the 1972–75 year-classes within a cluster and combined. Group I and II equal Cluster I and II, respectively.

temporal and spatial overlap between the clusters suggests the presence of gene flow, and concomitantly poorly isolated populations. However, it cannot be overstressed that we are not sure if any of the samples were obtained directly on spawning grounds, and none were composed of all ripe individuals. Therefore, such mechanisms as homing or habitat separation by time or depth may account for good reproductive isolation in the presence of temporal and spatial overlap.

Relationship Between Year-Class Strength and Degree of Heterozygosity

Measurements of the degree of heterozygosity, adjusted for gene frequency for the 1972 through 1975 year-classes, computed with the LDH and G-3-PDH data, within a cluster and combined, are depicted in Fig. 4. The large 1972 year-class (Humphreys 1978) contained an excess of heterozygotes, as expected from Castle–Hardy–Weinberg equilibrium, and the smaller 1973 through 1975 year-classes contained a deficit of heterozygotes. These results suggest that possibly larger year-classes are spawned when there is more interbreeding between populations. However, if the alleles have different fitnesses, the higher heterozygosity of the 1972 year-class may reflect a decrease in the intensity of selection due to a more favorable environment (Carson 1968; Ford 1971).

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Appendix

Estimation of MDH gamete frequencies by maximum likelihood based on the disomic inheritance model proposed in the text.

Phenotypes	S4	S3F	S2F2	SF3	F4	Total
Observed no.	A	B	C	D	E	N

Gametes	S1S2	S1F2	F1S2	F1F2
Frequencies	w	x	y	z

Let $a = A/N$, $b = B/N$, c $= C/N$, $d = D/N$, $e = E/N$.

Phenotypes	S4	S3F	S2F2	SF3	F4
Observed proportion	a	b	c	d	e
Expected proportion	w^2	$2w(x+y)$	$(x+y)^2 + 2wz$	$2z(x+y)$	z^2
$x + y =$ u yields	w^2	$2wu$	$u^2 + 2wz$	$2zu$	z^2

x and y are pooled because loci are not identifiable.

The likelihood equation is: the probability of observing A, B, C, D, or E given the parameters w, u, and z.

$$f(L) = (N!/(A!B!C!D!E!))(w^{2A+B})(z^{2E+D})(u^{B+D})(u^2 + 2wz)^C \times (2^{B+D}).$$

Let $K = (N!/(A!B!C!D!E!))(2^{B+D})$; therefore the log likelihood equation is

$$L = (2A + B)\log w + (2E + D)\log z + (B + D)\log u + C \log(u^2 + 2wz) + K.$$

Rewriting the likelihood equation using the observed proportion of phenotypes instead of the observed no. and ignoring K yields:

$$f(L') = (w^{2a+b})(z^{2e+d})(u^{b+d})(u^2 + 2wz)^c.$$

Then the log likelihood equation becomes

$$L' = (2a + b)\log w + (2e + d)\log z + (b + d)\log u + c \log(u^2 + 2wz).$$

Using the Lagrange method of undetermined multipliers because the function L' is bounded by the fact that $w + z + u$ must equal 1 the function L' becomes:

$$\phi = L' + \lambda(w + z + u - 1).$$

Equating the partial derivatives to zero gives:

(1) $\partial\phi/\partial w = (2a + b)/w + (2cz/(u^2 + 2wz)) + \lambda = 0$

(2) $\partial\phi/\partial z = (2e + d)/z + (2cw/(u^2 + 2wz)) + \lambda = 0$

(3) $\partial\phi/\partial u = (b + d)/u + (2cu/(u^2 + 2wz)) + \lambda = 0.$

Rewrite multiplying (1) by w, (2) by z, and (3) by u yields:

(4) $2a + b + (2czw/(u^2 + 2wz)) + \lambda w = 0$

(5) $2e + d + (2czw/(u^2 + 2wz)) + \lambda z = 0$

(6) $b + d + (2cu^2/(u^2 + 2wz)) + \lambda u = 0$

Adding these yields:

$$2(1 - c) + (2c(u^2 + 2wz))/(u^2 + 2wz) + \lambda(w + z + u) = 0.$$

Since $w + z + u = 1$, $\lambda = -2$, leaving the following:

(7) $2w = 2a + b + (2czw/(u^2 + 2wz))$

(8) $2z = 2e + d + (2czw/(u^2 + 2wz))$

(9) $2u = b + d + (2cu^2/(u^2 + 2wz))$.

Using $u = 1 - z - w$, we develop two independent equations:

(10) $(2w - 2a - b)((1 - z - w)^2 + 2wz) - 2czw = 0$

(11) $(2z - 2e - d)((1 - z - w)^2 + 2wz) - 2czw = 0.$

From (10) and (11) $2z = 2e + d - b - 2a + 2w$.

Substitution into (10) gives

$$(12)\ (2w - 2a - b)\left(\left(1 - w - e - \frac{d}{2} + \frac{b}{2} + a - w\right)^2 + w(2e + d - b - 2a + 2w)\right) - cw(2e + d - b - 2a + 2w) = 0.$$

Then solve for w, and finally:

(13) $z = w + 1/2(2e + d - b - 2a)$

(14) $u = 1 - z - w.$

A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater[1]

CAROL E. BOWER AND THOMAS HOLM-HANSEN[2]

Sea Research Foundation, Institute for Aquarium Studies, P.O. Box 7-495, West Hartford, CT 06107, USA

BOWER, C. E., AND T. HOLM-HANSEN. 1980. A salicylate–hypochlorite method for determining ammonia in seawater. Can. J. Fish. Aquat. Sci. 37: 794–798.

Total ammonia-nitrogen (NH_4-N) in artificial seawater was determined spectrophotometrically by a method in which indophenol blue is produced by the reaction of ammonia with salicylate and hypochlorite, in the presence of sodium nitroprusside. Recoveries of 10 to 1000 μg NH_4-N/L by the salicylate- and phenol–hypochlorite methods compared well (r = 0.99); the coefficient of variation for 25 μg NH_4-N/L and above was less than 3% for the salicylate–hypochlorite method. The new method is safer than the phenol–hypochlorite method because phenol is not used, but it may not be suitable for field determinations because of photosensitivity. The characteristic colors produced by increasing concentrations of ammonia make the assay useful for the direct, visual estimation of ammonia in culture systems.

Key words: ammonia, analytical methods, seawater analysis

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Nous avons déterminé l'azote ammoniacal total (NH_4-N) dans l'eau de mer artificielle par méthode spectrophotométrique dans laquelle la réaction de l'ammoniac avec le salicylate et l'hypochlorite en présence de nitroprussiate de sodium produit du bleu d'indophénol. Il y a bonne concordance entre les résultats obtenus par cette méthode et la méthode au phénol–hypochlorite dans la gamme de mesures de 10 à 1000 μg NH_4-N/L (r = 0,99); le coefficient de variation pour 25 μg NH_4-N/L et plus est inférieur à 3% avec la méthode au salicylate–hypochlorite. La nouvelle méthode est plus sûre que la méthode au phénol–hypochlorite parce qu'elle n'utilise pas de phénol, mais il se peut qu'à cause de sa photosensibilité elle ne convienne pas aux déterminations sur le terrain. Les couleurs caractéristiques produites par des concentrations croissantes d'ammoniac permettent l'estimation visuelle directe de l'ammoniac dans les aquariums marins.

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AMMONIA, the major nitrogenous excretory product of most aquatic animals (Forster and Goldstein 1969), can accumulate in culture systems lacking adequate or established biological filtration. The accurate determination of low concentrations of ammonia is particularly important in seawater culture, as ammonia is highly toxic to marine animals (Epifanio and Srna 1975; Wickins 1976; Delistraty et al. 1977; Armstrong et al. 1978).

The phenol–hypochlorite method of Solórzano (1969) is used widely to measure low concentrations of total ammonia-nitrogen (NH_4-N) in seawater. In this test, ammonia reacts with phenol and sodium hypochlorite in the presence of sodium nitroprusside, to form indophenol blue. The reaction pH is 9.8, and sodium citrate is used to prevent metal hydroxide precipitation. Inconsistencies with the phenol–hypochlorite method, resulting from photosensitivity and reagent instability, have been eliminated in recent modifications (Gravitz and Gleye 1975; Liddicoat et al. 1975; Hampson 1977). One remaining drawback, however, is that a noxious, highly volatile substance, tentatively identified as *o*-chlorophenol (Verdouw et al. 1978), is formed during the test. This compound penetrates human skin rapidly (Roberts et al. 1977) and is nearly three times more toxic to fish than phenol (Lammering and Burbank 1960). In a small culture facility with inadequate ventilation, we suspect that *o*-chlorophenol could contaminate recirculated aquarium water, in addition to posing a health hazard to laboratory workers.

When sodium salicylate is substituted for phenol, *o*-chlorophenol is not produced. A salicylate–hypochlorite method for determining serum ammonia was described by Reardon et al. (1966) and subsequently adapted for serum urea determination (Searcy et al. 1967) and automated Kjeldahl–nitrogen analysis (Bietz 1974; Conetta et al. 1976). Although Benesch and Mangelsdorf (1972) modified the original method for seawater determinations, we found that their method had lower

[1]Contribution No. 11, Sea Research Foundation, Inc.

[2]Present address (T.H.-H.): Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, Wash. 98105.

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sensitivity than the phenol–hypochlorite method and produced low recoveries of NH_4-N at concentrations above 100 μg/L. Verdouw et al. (1978) described a salicylate–hypochlorite method for measuring ammonia in freshwater; when we followed their recommendations for adapting the procedure for seawater analysis, we found substantial hydroxide precipitation and unsuitably high reagent blank absorbances (>0.05), which decreased the sensitivity of the test. The present study was conducted to determine the optimum physical and chemical conditions for determining ammonia in recirculated seawater by the salicylate–hypochlorite method. In the new procedure, phenol is not used as a reagent, volatile by-products are not generated, and this method is as sensitive as the phenol–hypochlorite method.

Materials

Reagents

Solutions were prepared volumetrically from analytical reagent grade chemicals, unless otherwise specified, using tap water passed through a mixed resin bed deionizing column (Crystalab DI-3) immediately before use.

Salicylate-catalyst solution — Four hundred and forty grams of sodium salicylate plus 0.28 g sodium nitroprusside/L. Store in brown glass bottle at 5°C. This solution darkens with age, leading to increased blank and sample absorbances, but is stable for at least 3 mo.

Alkaline–citrate solution — An amount of 18.5 g sodium hydroxide plus 100 g sodium citrate/L. Stable indefinitely.

Sodium hypochlorite solution — A stabilized commercial bleach, i.e. Clorox® (5.25% NaOCl) is suitable. Keep bottle stoppered tightly and store at 5°C.

Alkaline–hypochlorite solution — One volume of sodium hypochlorite solution diluted to 10 volumes with alkaline–citrate solution. Use within 1 h of preparation.

Reagent grade seawater (RSW) — An amount of 24.09 g sodium chloride, 10.91 g magnesium chloride hexahydrate, 4.02 g sodium sulfate (anhydrous), 1.5 g calcium chloride dihydrate, 0.68 g potassium chloride, and 0.20 g sodium bicarbonate/L. Salinity (S) is ~34‰. Adjust with deionized water to within 5‰ of sample salinities. Add 1 mL chloroform to preserve.

Ammonium sulfate standard solutions — Stock solution (100 mg NH_4-N/L) — An amount of 0.472 g ammonium sulfate/L. Add 1 mL chloroform to preserve, and store in brown glass bottle. *Working standard solution (10 mg NH_4-N/L)* — Dilute stock solution 1:10 with deionized water, add 1 mL chloroform/L and store in brown glass bottle.

Samples and Apparatus

Samples consisted of unfiltered artificial seawater (Instant Ocean®, $S = 30$‰) spiked with various concentrations of NH_4-N, or of water from recirculating seawater aquariums ($S = 28$ to 32‰) with established biological filtration. Newly mixed artificial seawater contained 100 to 200 μg NH_4-N/L, as determined with an Orion ammonia electrode by the standard addition method (Gilbert and Clay 1973). After recirculation for 2 wk in a mixing tank, ammonia levels decreased to a base of about 5 to 20 μg NH_4-N/L. Calibration standards consisted of 100 μg NH_4-N/L, prepared by dilution of the working standard solution with reagent grade seawater (RSW), and RSW ($S = 30$‰) served as the blank. Unless otherwise noted, determinations were carried out in triplicate, at ambient temperature (23 to 30°C), and under less than 34 lx of light from four 40-W fluorescent lamps at a distance of 2 m. Absorbances were measured in 1-cm cuvettes with a Turner model 330 spectrophotometer (bandwidth = 9 nm), using a No. 330-008 blocking filter. pH was determined with an Orion model 401 specific ion meter, using a glass pH electrode and a double-junction reference electrode (saturated KCl filling solution). An American Optical model 10419 hand refractometer was used for salinity determinations, and light was measured with a Weston Ranger 9 light meter.

Methods

The procedure for samples containing <1 mg NH_4-N/L is as follows. Transfer 25 mL of each sample, calibration standard, and blank solution to 50-mL erlenmeyer flasks. Add to each flask 3 mL salicylate–catalyst solution, mix contents, and then add 5 mL alkaline–citrate solution. Mix well, and stopper flasks. After 1 h, but before 3 h, read absorbances against reagent grade seawater at 640 nm, using a 1-cm light path. Samples should not be exposed to more than 85 lx of natural or artificial light during color development. Samples containing >1 mg NH_4-N/L should be diluted with RSW of the same salinity before reagents are added.

Sample absorbances were converted to concentrations of total ammonia-nitrogen by using the equation

$$\mu g\ NH_4\text{-}N/L = (E_s - E_b)F$$

in which E_s is the mean sample absorbance, E_b is the mean blank absorbance, and F is a conversion factor representing the quotient of the concentration of NH_4-N in the calibration standards (100 μg/L) and the corrected absorbance of the calibration standards, or:

$$F = 100/(E_{cs} - E_b),$$

where E_{cs} is the mean absorbance of the calibration standards. The concentration of unionized ammonia at various temperatures, pH values, and salinities can be calculated using the equations of Whitfield (1974, 1978) or from the tables of Bower and Bidwell (1978).

Results and Discussion

Effects of Reaction Conditions

A concentration of 0.0025% sodium nitroprusside in the reaction medium was sufficient to catalyze indophenol formation. This is about one-fourth the concentration used by Benesch and Mangelsdorf (1972) and one-eleventh that used by Searcy et al. (1967). Higher concentrations of catalyst produced a dark yellow reagent blank, which had unsatisfactorily high absorbance. Maximum color development was obtained with 4% sodium salicylate, the same final concentration used by Searcy et al. (1967) and Verdouw et al. (1978). Lower salicylate concentrations produced lower absorbances; this might explain the low absorb-

ances and recoveries of ammonia we found with the method of Benesch and Mangelsdorf (1972), in which only 1.3% salicylate was used. The final concentration of sodium hypochlorite used in our tests (0.08%) yielded more consistent recoveries than 0.02, 0.04, or 0.16% NaOCl. Absorbances were unchanged during 4 mo use of the same bottle of Clorox®, indicating that this commercial bleach is an acceptably stable chlorine source. Using the above reagent concentration, 60 min was required for full color development.

Conetta et al. (1976) and Pym and Milham (1976) reported that maximum color development in the salicylate–hypochlorite method occurs when the reaction pH is about 13. This value is somewhat higher than would be expected if, as reported by Verdouw et al. (1978), the pK_a of indophenol is 10.4. We found pH 13 impractical for seawater determinations, because reagent blanks absorbed too strongly, and also because it was difficult to include enough sodium citrate to prevent hydroxide precipitation. At the reaction pH of our tests, 11.6 ± 0.05, reagent blank absorbances were low (0.02–0.04 absorbance units, depending on the age of the salicylate–catalyst solution), and the sensitivity of the method was not lowered.

According to Pym and Milham (1976), there is an upward shift in the wavelength of maximum absorbance as reaction pH values fall below 13; at pH 11.6 in freshwater, maximum absorbance should occur at about 680 nm. In our seawater determinations, the wavelength of maximum absorbance at pH 11.6 was 640 nm. Samples prepared with deionized water and adjusted with HCl to a reaction pH of 11.6 absorbed most strongly at 655 nm, indicating that there may be a salt effect, as well as a pH effect, on the wavelength of maximum absorbance in the salicylate–hypochlorite method.

To assess the effect of salinity on absorbances, a concentrated reagent grade seawater ($S = 40‰$) was prepared and diluted to 20, 25, 30, and 35‰ with deionized water. Triplicate samples at each salinity and deionized water samples were spiked with ammonia to give concentrations of 100 μg NH_4-N/L; after treatment by the standard procedure, the absorbances of these samples and those of blanks at the same salinities were compared. As shown in Table 1, all absorbances increased with decreasing salinity, a phenomenon undoubtedly related to pH increases with successive dilutions. When seawater and deionized water samples were spiked with ammonia and adjusted to the same reaction pH, 11.6, the corrected absorbances for seawater were about 50% higher than those for deionized water, suggesting enhancement of color development by the ions present in seawater. This effect was not studied further, and we made no attempt to isolate the responsible ion or ions. Because of the concomitant effects of salinity decreases and pH increases on color development, however, it is clear that calibration should be carried out with reagent grade seawater adjusted to within 5‰ of sample salinities, or less, if a higher degree of precision is required.

TABLE 1. Effect of salinity on pH and absorbance in the salicylate–hypochlorite method. One hundred micrograms NH_4-N/L present in spiked samples. $n = 3$.

Salinity (‰)	pH	Absorbance[a]			F[b]
		E_s	E_b	$(E_s - E_b)$	
0	12.39	0.164	0.038	0.126	0.79
20	11.93	0.142	0.034	0.108	0.93
25	11.75	0.133	0.031	0.102	0.98
30	11.55	0.126	0.028	0.098	1.0
35	11.32	0.115	0.024	0.091	1.1
40	11.09	0.107	0.023	0.084	1.2

[a] E_s = mean sample absorbance; E_b = mean blank absorbance; $(E_s - E_b)$ = corrected absorbance.
[b] $F = 100/(E_s - E_b)$.

McCarthy and Kamykowski (1972) and Gravitz and Gleye (1975) found that color development and stability in the phenol–hypochlorite method are affected adversely by exposure of samples to bright light. Subsequently, Liddicoat et al. (1975) and Hampson (1977) reported optimal color development after the irradiation of samples with long-wave ultraviolet light, and Patton and Crouch (1977) explained the mechanism of the ultraviolet effect. The effects on indophenol formation of wavelengths outside the ultraviolet range apparently have not been studied.

To determine the influence of light intensity on the salicylate–hypochlorite reaction, we spiked a series of seawater samples with 100 μg NH_4-N/L and measured absorbances after 1, 3, and 5 h of color development in the dark (<0.03 lx), under fluorescent light (34 or 85 lx), and in filtered sunlight (170, 340, or 685 lx). Contrary to the findings of Verdouw et al. (1978) that poor color development occurs in the dark, we found no difference between the absorbances of samples held in darkness and under less than 85 lx. Moreover, samples exposed to 170 lx and more absorbed progressively less strongly, with exposure to 685 lx (the equivalent of bright shade) producing absorbances 22% lower than dark controls at 1 h, 50% lower at 3 h, and 70% lower at 5 h. The absorbances of all samples decreased with time; those of samples exposed to <85 lx had decreased about 2% between 1 and 3 h, and about 6% by 5 h. Sodium salicylate is light-sensitive; this may be the cause of decreased absorbances of samples after prolonged exposure to high light levels. Sample flasks should therefore not be exposed to more than 85 lx of light during color development, and absorbances should be read within 3 h.

ACCURACY AND REPRODUCIBILITY

Newly mixed artificial seawater and seawater from

TABLE 2. Accuracy and reproducibility of the salicylate hypochlorite method for $n = 3$.

Concentration NH_4-N (μg/L) added	Trial No.[a]	Mean absorbance ±SD (%)	Recovery μg NH_4-N/L	Recovery %
0 (Blank)	1	0.052±0.004 (7.7)	—	—
	2	0.127±0.002 (1.6)	—	—
10	1	0.063±0.003 (4.8)	11.7	117
	2	0.136±0.004 (2.9)	9.8	98
25	1	0.075±0.001 (1.3)	24.4	98
	2	0.152±0.002 (1.3)	25.3	103
50	1	0.100±0.001 (1.0)	49.6	99
	2	0.179±0.001 (0.6)	53.3	107
100 (Standard)	1	0.148±0.003 (2.0)	—	—
	2	0.225±0.004 (1.8)	—	—
200	1	0.252±0.004 (1.6)	208	104
	2	0.320±0 (0)	196	98
500	1	0.540±0.016 (3.0)	505	101
	2	0.607±0.006 (1.0)	487	97
1000	1	1.01±0 (0)	990	99
	2	1.03±0.01 (1.0)	913	91

[a]Trial 1 carried out with recirculated seawater from a tank with established biological filtration; trial 2 with newly mixed artificial seawater.

an aquarium with biological filtration was spiked with ammonia to give final concentrations of 10 to 1000 μg NH_4-N/L, and unspiked seawater was used for reagent blanks. The results are given in Table 2. The recoveries of ammonia indicate high accuracy, and the low standard deviations (coefficient of variation <3% for concentrations above 25 μg NH_4-N/L) indicate good reproducibility. The factor relating absorbance to concentration in μg/L was consistently 1.0 for salinities of 28 to 32‰.

To compare the salicylate–hypochlorite method with the phenol–hypochlorite method, seawater from established culture systems and from a mixing tank was analyzed by the standard salicylate procedure and by the method of Solórzano (1969), before and after spiking with various concentrations of ammonia. Calibration was made with RSW and deionized water for the salicylate– and phenol–hypochlorite methods, respectively. As shown in Table 3, there was excellent correlation between ammonia recoveries by the two methods ($r = 0.99$).

Relationship Between Visible Colors and Ammonia Concentration

The visible colors of samples treated by the phenol–hypochlorite method range from light blue to dark blue,

TABLE 3. Comparison of the phenol–hypochlorite and salicylate–hypochlorite methods for ammonia determination. $n = 2$.

Sample No.[a]	Concentration spiked (μg NH_4-N/L)	μg NH_4-N/L found (% recovery)[b] Phenol method	Salicylate method	Difference (μg NH_4-N/L)
1	0	5.6	6.5	0.9
	10	17 (113)	18 (114)	1
2	0	2.4	3.5	1
	50	56 (106)	54 (101)	2
3	0	15	20	5
	100	134 (119)	129 (109)	5
4	0	147	137	10
	500	656 (102)	623 (97)	33

[a]Samples 1 and 2 were seawater from culture tanks with established biological filtration; samples 3 and 4 were freshly mixed hw-Marinemix® and Instant Ocean®, respectively.
[b]$F = 0.81$ (phenol method); $F = 1.0$ (salicylate method).

depending on the concentration of ammonia present. With the salicylate–hypochlorite method, distinct color changes correspond with increasing ammonia concentrations as follows:

Concentration NH_4-N in sample (μg/L)	Color
0	Light yellow
10	Light yellow-green
100	Green
500	Blue-green
1000	Blue

The salicylate–hypochlorite method thus provides a simple and direct means for visual spot checks, without any calibration or instrumentation, on ammonia levels in heavily stocked culture systems and on the progress of the nitrification sequence in tanks without established biological filtration.

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Viral Erythrocytic Necrosis: Some Physiological Consequences of Infection in Chum Salmon (*Oncorhynchus keta*)

JOHN R. MACMILLAN AND DAN MULCAHY

National Fisheries Research Center, Bldg. 204, NSA, Seattle, WA 98115, USA

AND MARSHA LANDOLT

College of Fisheries, University of Washington, Seattle, WA 98195, USA

MACMILLAN, J. R., D. MULCAHY, AND M. LANDOLT. 1980. Viral erythrocytic necrosis: some physiological consequences of infection in chum salmon (*Oncorhynchus keta*). Can. J. Fish. Aquat. Sci. 37: 799–804.

Erythroid cells in chum salmon (*Oncorhynchus keta*) susceptible to infection with erythrocytic necrosis virus (ENV) were examined by light and electron microscopy. Cells of stages II, III, IV, V, and VI contained complete eyrthrocytic necrosis virions in the cytoplasm. Viruses closely resembling ENV were also detected in the nuclei of some erythroblasts. Some secondary consequences of ENV infection were a threefold greater mortality rate from vibriosis, a significantly decreased tolerance to oxygen depletion, and a decreased ability to regulate serum sodium and potassium in saltwater.

Key words: viral erythrocytic necrosis, erythroblasts, pathogenesis

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Nous avons examiné au microscope photonique et électronique des érythrocytes de saumon keta (*Oncorhynchus keta*) susceptibles à l'infection par le virus de la nécrose érythrocytaire (ENV). Les cellules aux stades II, III, IV, V et VI contiennent dans leur cytoplasme des virions complets de la nécrose érythrocytaire. Nous avons également détecté, dans les noyaux de certains érythroblastes, des virus ressemblant étroitement à celui de l'ENV. Comme conséquences secondaires de l'infection par ENV, on note un taux de mortalité trois fois supérieur due à la vibriose, une tolérance nettement inférieure à l'épuisement d'oxygène et enfin, une aptitude moindre à régler le sodium et le potassium sérique dans l'eau salée.

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THE etiologic agent of viral erythrocytic necrosis (VEN) of fishes has been identified as a virus tentatively classified in the Iridoviridae (Reno et al. 1978). The virus infects the erythrocytes of several species (Appy et al. 1976; Walker and Sherburne 1977; Evelyn and Traxler 1978; MacMillan and Mulcahy 1979) and may be related to erythrocytic viruses reported in the leopard frog, *Rana pipiens* (Bernard et al. 1968), and gecko, *Gehyra variegata* (Stehbens and Johnston 1966). The virus infection produces a severe, chronic anemia in which the hematocrit may approach 2% (Evelyn and Traxler 1978; MacMillan and Mulcahy 1979); yet, little is known about the viral pathogenesis in fish.

Unlike fish virus infections such as infectious hematopoietic necrosis, VEN has not yet been associated with massive mortalities. Evelyn and Traxler (1978) reported mortalities up to 0.3% per day in chum salmon (*Oncorhynchus keta*) attributable to VEN. Sherburne (1977) reported VEN in the alewife (*Alosa pseudoharengus*) and hypothesized a deleterious effect of virus infection based on incidence data, as did MacMillan and Mulcahy (1979).

The research reported here documents some physiological consequences of erythrocytic necrosis virus (ENV) infection in chum salmon and further examines the pathogenesis of this viral disease. Erythroid cells susceptible to viral infection were determined during experimentally induced disease and virus-infected chum salmon were exposed to hypoxic conditions, a secondary pathogen, and saltwater.

Materials and Methods

Erythrocytic necrosis virus was obtained as described by MacMillan and Mulcahy (1979). Chum salmon blood was identified as ENV-positive or -negative from a peripheral blood smear. A heavily infected sample was frozen, thawed, sonicated, and filtered through a 0.22-μm cellulose filter (Nucleopore Corp.). The filtrate was diluted 1:10 with phosphate-buffered saline (pH 7.0) and 0.05 mL was injected intraperitoneally into anesthetized fish (1–4 g

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TABLE 1. Classification stages used to identify chum salmon erythroblasts. Leishman–Giemsa stain, 1000×.

Stage	Diameter (μm) (stage IV–VI dimensions)	Staining	Nuclear/cytoplasmic ratio
I	16–18	Leptochromatic lightly eosinophilic nucleus and darkly basophilic cytoplasm Occasionally one or several acidophilic nucleoli	9/10
II	16	Pachychromatic eosinophilic nucleus; deep blue cytoplasm often with a perinuclear halo	4/5
III	12	Pachychromatic darkly eosinophilic nucleus with light blue cytoplasm and perinuclear halo	2/3
IV	Elliptical mature morphology 15 × 9	Reticulated deeply eosinophilic nucleus, gray cytoplasm	2/3
V	Elliptical mature morphology 15 × 9	Dark purple reticulated nucleus with light gray to lavender cytoplasm	2/3
VI	Mature cell 15 × 9	Basophilic condensed nucleus with a reticulated pattern Eosinophilic cytoplasm	2/3

wet wt). Control fish were injected with similarly prepared ENV-negative blood. After injections all fish were held for 3 wk before use in experiments. Previous data (MacMillan and Mulcahy 1979) indicated that erythroblasts were prominent after this period and that viral cytoplasmic inclusions would be present in the circulating erythroblasts. The salmon were maintained in freshwater at 14°C and fed daily with Oregon Moist Pellet Formula II (Moore-Clarke Co.).

Erythroid Cells Susceptible to Infection

To prepare tissues for electron microscopy, we cut the pronephros and spleen into 1-mm pieces and placed them in cold (4°C) Trump's fixative (McDowell and Trump 1976). The procedure of Reno et al. (1978) was then followed, except that Epon 812 was used. Sections were examined on a JEOL 100 B electron microscope.

Blood smears and imprints from the kidney and spleen were taken from each fish used for electron microscopy. Preparations were stained with Leishman–Giemsa (Wedemeyer and Yasutake 1977) and examined for viral cytoplasmic inclusions. Erythroid cell developmental stages were identified by using criteria for higher vertebrates (Lessin and Bessis 1977) but modified for chum salmon (Table 1) on the basis of hemoglobin incorporation of ^{55}Fe (J. R. MacMillan et al. unpublished data).

Susceptibility to Vibrio Anguillarum

Vibrio anguillarum isolate LSI-74 (from Waldport, OR) was prepared as described by Udey (1978). A bacterial suspension of 2×10^4 cells/mL was prepared in 14°C water. One litre of this suspension was placed in each of six 8-L tanks containing either 10 ENV-infected fish or 10 control fish. After a 30-min exposure, normal water flow was resumed. Mortalities were assessed daily for 20 d and the kidney cultured for *V. anguillarum*. Significant differences between mortality rates were detected by using Bonferoni *t*-statistics (Gill 1978).

Resistance to Oxygen Depletion

We evaluated resistance of ENV-infected and noninfected fish to oxygen depletion by using a sealed jar bioassay (Carter 1962). The dissolved oxygen concentration present when fish ceased opercular movement (point of death) was determined by the modified Winkler method (American Public Health Association 1971). Fish weighed 3.0 ± 0.5 g and had been infected with ENV for variable lengths of time to induce different degrees of anemia and erythroblastosis (MacMillan and Mulcahy 1979). Jars were submerged in a water trough holding circulating 14°C water. A single diseased or control fish was placed in a jar holding oxygen-saturated water (10.4 mg/L). Thirty replicates were made. At the point of death blood smears were made, fixed in 100% methanol, and stained with Leishman–Giemsa stain. Hematocrits were determined from blood collected in a microhematocrit tube and centrifuged at 17 000 × *g* for 5 min.

Seawater Challenge

Two aquaria, filled with a 30‰ saline solution (Instant Ocean, Aquarium Systems, Inc.) and with constant aeration, were placed in a tank containing circulating 14°C water. One aquarium held 25 virus-infected fish and the other 25 control fish. Five fish were selected from each group daily and killed with a blow to the head. Blood smears were made and hematocrits were determined. Each microhematocrit tube was broken at the serum–erythrocyte boundary and the serum frozen at −70°C. We determined serum Na^+ and K^+ concentrations using flame spectrophotometry, and an analysis of variance was performed between infected and control fish.

Results and Discussion

Erythroid Cells Susceptible to Infection

Viral cytoplasmic inclusion bodies were observed in

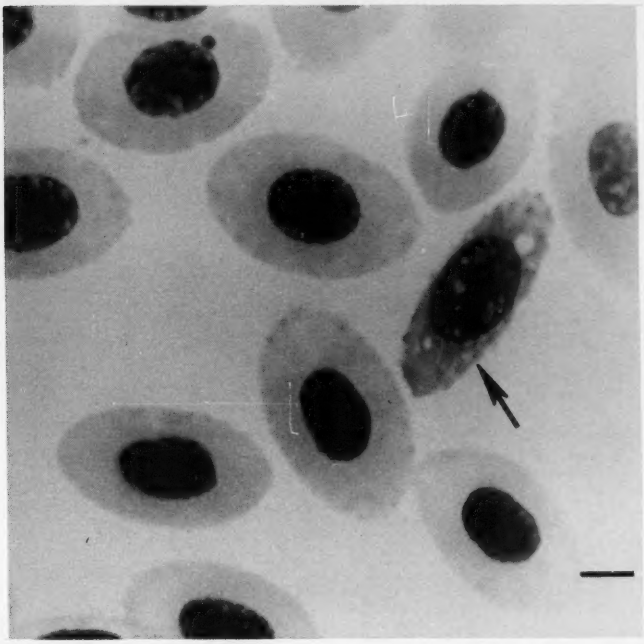

FIG. 1. Circulation blood smear showing erythrocytic cytonuclear dissociation. Leishman–Giemsa stain. Bar represents 5 μm.

all morphologically definable stages of erythrocyte development observed with the light microscope. Electron micrographs of the anterior kidney showed intact virions in the cytoplasm of early erythroblasts. These observations suggest that all stages of erythroid cells were suceptible to viral infection. Severe anemia may be a prominent characteristic associated with VEN (Evelyn and Traxler 1978; MacMillan and Mulcahy 1979). The reactive erythroblastosis in which 100% of the erythroid cells are erythroblasts (MacMillan and Mulcahy 1979) probably indicates a demand for oxygen-carrying erythrocytes. This would be met by a premature release of erythroid cells.

Cytonuclear dissociation (Fig. 1) was often observed by light microscopy in the erythroblasts of infected fish. These cells had a nearly mature morphology but with a basophilic cytoplasm. The asynchronous development may result directly from viral infection or from an aberrant cellular regulation system indirectly attributable to ENV.

Mature virions were occasionally observed ultrastructurally in the nucleus of early erythroblasts (Fig. 2) and had the same size and morphology as those described by Evelyn and Traxler (1978) and MacMillan and Mulcahy (1979) for ENV in chum salmon. Intranuclear inclusions were occasionally observed with light microscopy, but the relationship of these inclusions to the virions observed by electron microscopy remains obscure. In as much as naturally occurring multiple virus infections have been reported in fish (Schlotfeldt and Frost 1975; Mulcahy and Fryer 1976) and the artificial infections reported here were established with crude unpurified material from naturally infected fish, a true association of the virus appearing in the nucleus with VEN disease was not proved. Determination of whether that association exists must await the use of isolated and cloned ENV to establish infections.

Susceptibility to *Vibrio Anguillarum*

The mortality rate following challenge with *V. anguillarum* was much higher in fish with VEN than in control fish (Fig. 3). Since there was no significant difference ($P = 0.05$) within groups, the data were pooled. Fish with VEN averaged 2.6 times greater mortality from *V. anguillarum* than control fish and showed a shorter time-to-death after bacterial exposure. Reisolation of *V. anguillarum* was possible in 98% of the fish with VEN but in only 5% of the control fish.

Whereas long-term VEN infections cause severe erythroblastosis that results in a macrocytic hypo-

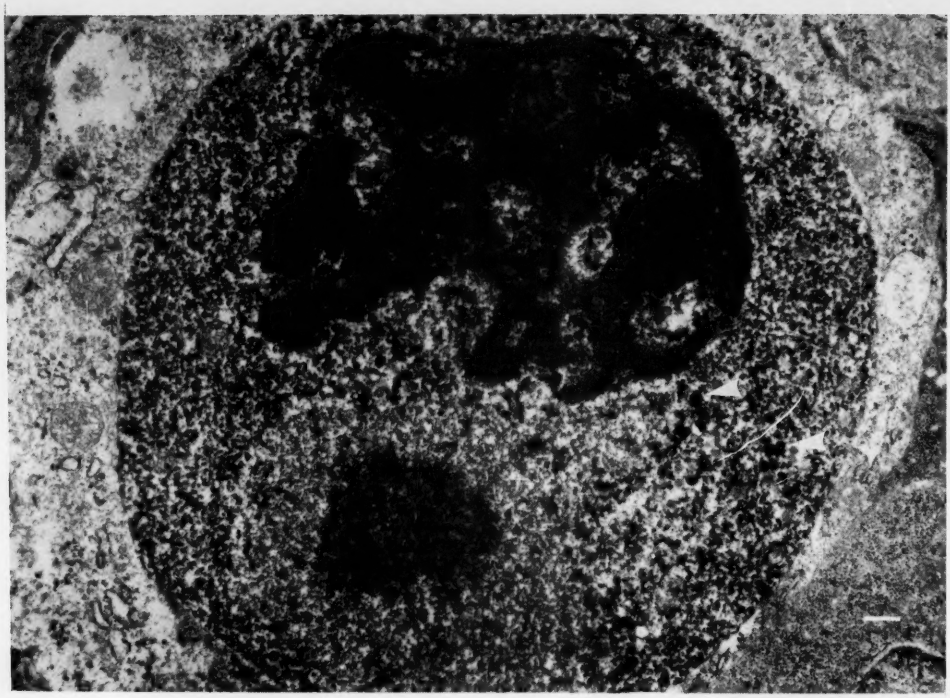

FIG. 2. Electron micrograph of stage I or II erythroblast with intranuclear VEN-like virions (arrows). Bar represents 0.05 μm.

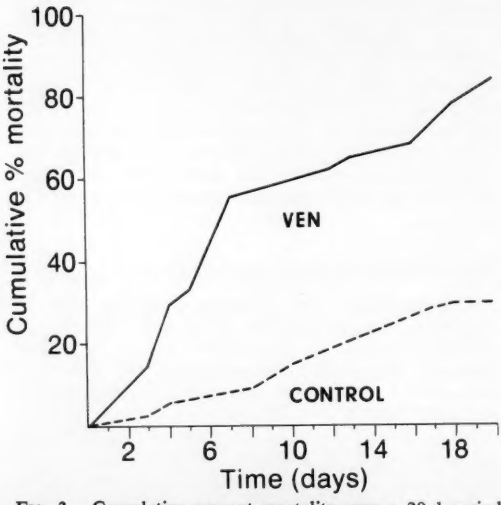

FIG. 3. Cumulative percent mortality over a 20-d period in VEN-diseased fish and controls, both with vibriosis.

chromic anemia, vibriosis causes an acute, hemolytic, microcytic, normochromic anemia perhaps induced by a potent hemolytic toxin (Wolke 1975). It is unknown whether there is an interplay between ineffective erythropoiesis and shortened erythrocyte survival; however, the greater mortality of ENV-infected fish reported here may be due to the synergism of the two anemia-inducing agents.

RESISTANCE TO OXYGEN DEPLETION

The oxygen concentration present when ENV-infected fish died was inversely correlated ($r = 0.80$) with the hematocrit (Fig. 4). Control fish (mean hematocrit 31%) ceased opercular movement only at low oxygen concentrations (1.6 mg/L ± 0.3 SD) whereas diseased fish (mean hematocrit 15%) died at a significantly higher ($P = 0.05$) oxygen concentration (3.9 mg/L ± 0.9 SD). Fish with VEN dying at a higher oxygen concentration (3.5–4.5 mg/L) but with a hematocrit of 20% had severe erythroblastosis which accounted for the relatively elevated hematocrits. Thorling and Erslev (1968) observed that anemia was associated with a decrease in the tissue oxygen tension

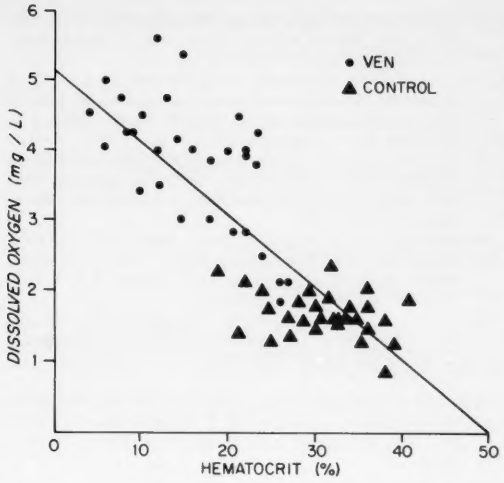

FIG. 4. Hematocrits compared with dissolved oxygen concentration at the point of death for VEN-diseased and control fish.

proportional to the degree of anemia. The mechanism in VEN-diseased fish may be similar. A decreased number of oxygen-carrying erythrocytes in conjunction with erythroblastosis decreases oxygen transport and may significantly impair fish health. Survival of ENV-infected fish may be severely decreased by environmental perturbations that deplete ambient oxygen and the oxygen demands required during predator avoidance and migration.

SEAWATER CHALLENGE

Entry of chum salmon into saltwater environments may occur soon after emergence from the spawning redds. Impaired osmoregulatory capability may prevent survival in the sea or, conversely, limit spawning by returning adults. The wide range of SD values for serum Na^+ levels obtained for VEN-infected fish, although not significantly different ($P \leq 0.05$), contrasts with the narrow range for control fish (Table 2). Serum Na^+ and K^+ values determined during the seawater challenge suggests that fish with VEN have osmoregulatory difficulty; however, its effect on survival is unknown.

Though large-scale mortalities directly attributable to VEN have not been reported, our results indicate that the disease could have a significant impact on survival. The interactions of pathogen, fish, and the environment determine the pathological manifestations of disease (Snieszko 1974; Wedemeyer et al. 1976). The increased susceptibility of VEN-diseased fish to environmental stress and a secondary pathogen suggests the importance of this interaction in limiting the survival of fish in the wild.

TABLE 2. Comparison of serum sodium and potassium concentrations and hematocrits from ENV-infected and control chum salmon during a 5-d seawater challenge test, 30‰ NaCl, 14°C.[a]

Category of fish and day	Hematocrit (%)	Na^+ (mol/L)	K^+ (mol/L)
VEN infected			
1	8.4±3.4	139.6±22.1	9.5±2.4
2	5.3±3.6	131.2±42.7	7.1±1.3
3	6.3±1.8	168.2±10.3	7.7±3.2
4	9.8±5.5	155.9±30.6	6.8±1.9
5	9.3±3.3	167.9±5.8	5.9±0.9
Control			
1	33.4±5.8	151.2±1.9	9.0±0.2
2	33.8±5.4	141.2±20.3	6.9±1.8
3	28.0±3.7	153:4±5.1	6.5±1.2
4	31.2±6.6	155.2±5.5	6.8±1.9
5	29.0±4.7	152.2±4.6	6.9±1.2

[a]Each value represents $\bar{X} \pm$ SD for five fish.

Acknowledgments

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Plasma Cortisol Levels of Fingerling Rainbow Trout (*Salmo gairdneri*) at Rest, and Subjected to Handling, Confinement, Transport, and Stocking

BRUCE A. BARTON[1]

Fish and Wildlife Division, Alberta Energy and Natural Resources, Sam Livingston Fish Hatchery, Calgary, Alta. T2G 4T9

AND

RICHARD E. PETER AND CHRISTINE R. PAULENCU

Department of Zoology, University of Alberta, Edmonton, Alta. T6G 2E9

BARTON, B. A., R. E. PETER, AND C. R. PAULENCU. 1980. Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37: 805–811.

Fingerling rainbow trout, acclimated to hatchery raceways or laboratory aquaria, had low plasma cortisol levels ($\leq$2 ng/mL), with no apparent daily cycle in levels. Netting of cohorts out of an aquarium, without agitation, did not cause a rise in plasma cortisol concentrations in remaining fish. However, following 90 s of handling and confinement by netting, fingerlings had a sharp rise in plasma cortisol to a peak at 15 min, and then a gradual decline to the basal level over 2 h. Gentle agitation and intermittent restraint with a dip net in the aquarium caused a gradual increase in plasma cortisol concentrations. Intense handling and severe confinement caused a rapid increase in plasma cortisol to a plateau, two to four times greater than the peak levels found in fingerlings subjected to the less vigorous stressors; high levels of plasma cortisol were maintained to the median tolerance limit. During a stocking operation, there was a rapid rise in plasma cortisol concentrations during the initial capture from the rearing ponds, and high levels were maintained through to stocking into the lake 6 h and 10 min later, although a small decrease occurred during transit while the fish were in the truck tanks. After stocking, plasma cortisol concentrations in caged fingerlings did not decrease to the basal level until 8 d poststocking.

Key words: rainbow trout, fingerlings, plasma cortisol, cycles, handling stress, stocking stress

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Des fingerlings de truite brune, acclimatés à des dalots d'écloserie ou à des aquariums de laboratoire, ont de bas niveaux de cortisol plasmatique ($\leq$2 ng/mL), sans que les niveaux accusent de cycle nycthéméral apparent. L'enlèvement au filet de cohortes de l'aquarium, sans agitation, ne cause pas d'augmentation des concentrations de cortisol plasmatique chez les poissons laissés dans l'aquarium. Cependant, après 90 s de manipulation et d'emprisonnement dans le filet, les fingerlings subissent une montée rapide de cortisol plasmatique vers un pic à 15 min et un déclin graduel vers le niveau de base après 2 h. Une légère agitation et la retenue intermittente dans l'épuisette dans l'aquarium produit un accroissement graduel des concentrations de cortisol plasmatique. La manipulation et l'emprisonnement violents résultent en une augmentation rapide du cortisol plasmatique vers un plateau qui est de deux à quatre fois plus élevé que les niveaux de pointe observés chez des fingerlings soumis aux contraintes moins rigoureuses; les hauts niveaux de cortisol plasmatique se maintiennent à la limite de tolérance médiane. Lors d'une opération d'ensemencement, il y eut élévation rapide des concentrations de cortisol plasmatique au moment de la capture initiale dans les étangs d'élévage. Ces hauts niveaux persistèrent jusqu'au moment de la mise en liberté, 6 h et 10 min plus tard, bien qu'il se soit produit une faible diminution durant le transport, alors que les poissons étaient dans les réservoirs du camion. Après ensemencement, les concentrations de cortisol plasmatique chez des fingerlings maintenus dans des cages ne se rétablirent pas au niveau de base avant 8 jours après ensemencement.

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[1]Author to whom correspondence should be sent.

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THE elevation of plasma cortisol in fish as an indicator of stress resulting from various capture, handling, and holding practices is well established (LeLoup-Hatey 1964; Hane et al. 1966; Fagerlund 1967; Fuller et al. 1974; Spieler 1974; Fryer 1975; Mazeaud et al. 1977; Strange et al. 1977, 1978; Strange and Schreck 1978). Although plasma cortisol levels, under various conditions, in yearling and larger rainbow trout (*Salmo gairdneri*) have been investigated (Hane and Robertson 1959; Hane et al. 1966; Donaldson and McBride 1967; Hill and Fromm 1968; Wedemeyer 1970), there are no reports on the plasma cortisol concentrations in hatchery-reared fingerling rainbow trout. Similarly, information is lacking on the diurnal variations in plasma cortisol levels in unstressed rainbow trout, although this aspect has been studied for several nonsalmonid fishes (Boehlke et al. 1966; Srivastava and Meier 1972; Garcia and Meier 1973; Redgate 1974; Fryer 1975; Meier and Srivastava 1975; Singley and Chavin 1975; Peter et al. 1978). Establishment of the diurnal baseline of plasma cortisol concentrations is necessary if the elevations resulting from various stressors are to be assessed.

In a typical stocking operation, fingerling fish are captured in the hatchery, loaded into tank trucks, transported, and subsequently planted directly into a lake or stream. In addition to other surrounding physical and chemical changes, fish are subjected to varying degrees of handling and confinement during these procedures. These stressors could result in reduced fitness of the fish (Strange and Schreck 1978) and may be partly responsible for previously observed high seasonal poststocking mortality (Ayles et al. 1976) by increasing vulnerability to additional stressors.

One objective of these experiments was to establish the diurnal resting levels of plasma cortisol in fingerling rainbow trout. A second objective was to determine the stress-elevated plasma cortisol concentrations in fingerling rainbow trout subjected to brief handling and confinement, continuous mild handling, and continuous handling and confinement severe enough to cause mortality. The third and most important purpose of this study was to monitor fingerling rainbow trout during a normal hatchery stocking operation to discern the level of stress and the time for recovery from stress, as reflected by changes in plasma cortisol concentrations.

Materials and Methods

Fingerling (0+ yr) rainbow trout (*Salmo gairdneri*) were supplied by the Sam Livingston Fish Hatchery, Calgary, from eggs obtained from fall spawning brood stock (Mt. Lassen Trout Farms, CA). Both hatchery fish and tank acclimated fish were fed regularly with commercial trout food (Silver Cup®) at ~1% body weight/d.

EXPERIMENTAL CONDITIONS

Daily variations in plasma cortisol — Hatchery fish, in raceways at ~20 g (0.6–0.7 fish)/L, at the time of year of experiment (July 5, 1978) were under a natural photoperiod of 16 h light and 8 h dark (16L:8D), with 0.5–1 h of twilight at each transition. Surface illumination at water level in the raceways during light period ranged from 20 to 270 lx. During the experiment, dissolved oxygen (DO) was 8.3–8.8 mg/L, total dissolved solids (TDS) was 330–380 mg/L, pH was 7.2–7.3, and the temperature was 11.5–12.0°C. At 3-h time intervals over a 27-h period, ~20 fish (mean fork length (FL) 130 mm, and mean body weight (wt) 28.7 g) were quickly dip-netted from the same raceway for analysis.

In the laboratory, ~300 rainbow trout fingerlings (mean FL 112 mm, mean body wt 17.1 g) were acclimated in a 492-L recirculating tank supplied with aerated well water at 11–13 L/min, for 4 wk at 0.6 fish/L. The 15L:9D photoperiod was provided with a 15-W incandescent bulb adjusted to provide 100–110 lx surface illumination at water level. Throughout the acclimation period and experiment, DO was 7.7–7.9 mg/L, TDS was 330–340 mg/L, pH was 7.5, and the temperature was 11.0°C. Twelve fish were dip-netted from the tank for analysis at 9-h intervals until 10 samples were taken; the sample points were reassembled to give 3-h time points over a 27-h period.

Brief handling and confinement, and removal of cohorts — Approximately 100 fingerlings (mean FL 127 mm, mean body wt 21.9 g), acclimated at 0.4 fish/L for 3 wk, were subjected to brief handling and confinement by netting all the fish from the aquarium and transferring them to a 25-L tub. After 90 s at 5.0 fish/L, the fish were returned to their home tank. Before handling ($t = 0$), and 5, 15, and 30 min, and 1, 2, and 4 h after the stress period, eight fish were dip-netted from the aquarium for analysis. At $t = 0$ DO was 7.2 mg/L, TDS was 325 mg/L, pH was 7.6, and the temperature was 12.0°C.

For control, an additional group of fingerlings (mean FL 127 mm, mean body wt 23.5 g) were acclimated and sampled in the same manner as described above, except that the 90-s handling and confinement stress was omitted. At $t = 0$ DO was 7.0 mg/L, TDS was 340 mg/L, pH was 7.6, and the temperature was 10.5°C.

Continuous mild handling — Approximately 350 fingerlings, acclimated for 2 wk at 0.4 fish/L, were exposed to continuous mild agitation and repeated capture–release within their home aquarium for 1 h. At $t = 0$, and at 5-, 15-, 30-, and 60-min intervals during this period, seven to eight fish (not measured) were removed for analysis. At $t = 0$ DO was 6.8 mg/L, TDS was 280 mg/L, pH was 7.4, and the temperature was 8.3°C.

Continuous intense handling and severe confinement to TLm — Fingerlings ($n = 311$, mean FL 114 mm, mean body wt 17.1 g), acclimated for 7 d at 0.4 fish/L, were subjected to continuous severe confinement by transferring the fish with dip net to a 30-cm-diameter cylindrical cage immersed to 30 cm in the tank, such that all fish were touching each other. Additional handling was applied by stirring the confined fish with a hand net every 10 to 20 min. Before and throughout the experiment DO was 5.5–6.2 mg/L, TDS was 340–350 mg/L, pH was 7.2–7.3, and the temperature was 8.8–9.1°C.

At $t = 0$, and at 0.5, 1, 2, 3, 4 h and at 2- or 3-h intervals until the approximate median tolerance limit (TLm, i.e. 50% mortality) of remaining fish was reached, 12 finger-

lings were removed for analysis. As fish were removed, the depth of cage immersion was decreased so that the density of 15 to 16 fish/L remained relatively constant.

Transport and stocking — In the loading and stocking operation, two 0.9-m × 3.6-m-diameter rearing ponds, containing ~3700 fingerlings (mean FL 137 mm, mean body wt 30.9 g) at 0.4 fish/L, were drawn down while all fish were captured with dip nets and loaded into hatchery truck tanks at 2.0 fish/L. Fingerlings were shipped and subsequently planted by hand into a 16-ha (1 ha = 10 000 m²) lake. At stocking, subsamples of 14 fish were placed into each of six 60-cm-length by 30-cm-diameter live cages, anchored 1–1.5 m off the bottom of the lake. In the rearing ponds DO was 7.4 mg/L, TDS was 375 mg/L, pH was 7.4, and the temperature was 10.5°C. In the truck tanks DO was 11.6–15.4 mg/L, TDS was 370 mg/L, pH was 6.7–7.0, and the temperature was 9.8–11.1°C. At the lake DO was 9.2–9.8 mg/L, TDS was 270 mg/L, pH was 8.1–8.3, and the temperature was 19.2–23.0°C during the experiment.

At $t = 0$, before disturbance, 12 (total) fingerlings were removed from each pond for analysis ("initial" sample). At 35 and 55 min after initiating capture from the hatchery ponds, six fish were sampled from each pond, respectively ("capture" sample). At 1 h 20 min, when all the fish were loaded into the truck tanks ("truck" sample), and at 3 h 40 min (2.5 h in transit, "transit" sample) and 6 h 10 min (arrival at lake and start of stocking, "stocking" sample), 12 fish were sampled from the truck tanks for analysis. At 1, 2, 4, 8, 11, and 14 d after planting, fingerlings were sampled from the live cages by rapidly removing a single cage with fish on each day and immediately immersing it into 10 L of anesthetic solution.

Blood sampling and cortisol analysis — To obtain blood, sampled fingerlings were rapidly anesthetized in 200 mg/L tricaine methanesulfonate solution neutralized with about 5 mL 0.1 mol/L NaOH. Immobilization was complete within 30 s of capture. A blood sample of 0.1–0.2 mL was withdrawn from the caudal vasculature, using a 27-gauge needle attached to a heparinized 1-mL disposable syringe. Fish were weighed, fork length measured, and sacrificed after blood removal. After 5 min centrifugation (Beckman Microfuge), plasma samples were extracted and stored at −21°C for cortisol determination by radioimmunoassay (RIA).

Cortisol is the predominant corticosteroid in rainbow trout (Idler and Truscott 1972). Cortisol RIA was done with commercially available kits (Cortisol Autopak® ^{125}I RIA kit, Micromedic Systems, Inc.). The assay involves incubation at 37°C for 1 h of a 5-μL volume of plasma with ^{125}I-cortisol in 1.0 mL of phosphate-buffered saline (pH 7.5), in assay tubes coated with a gel preparation containing an antibody highly specific to cortisol. The contents of the tubes were then aspirated, and the tubes washed twice with double-distilled water, after which the bound radioactivity was counted in a well scintillation counter. All samples were assayed in duplicate. With this assay there is parallelism between cortisol standards and dilutions of rainbow trout or goldfish plasma. This assay is based on a similar antibody to cortisol as in the assay described by Peter et al. (1978), and samples have similar readings in each assay system. The performance characteristics of this cortisol RIA are as follows: within-assay variability, defined as the coefficient of variation (CV) of the cortisol level measured in a sample assayed six times in one assay, 0.04; between-assay variability, defined as the CV of the cortisol levels measured in a single sample assayed in six different assays, 0.12; minimum level of sensitivity, defined as the level 2 SD away from the zero dose measurement, 2.0 ng cortisol/mL (samples with levels indicated below the minimum level of sensitivity were assigned a value of zero). Analysis of variance and Duncan's new multiple-range test at $P < 0.05$ (Steel and Torrie 1960) were conducted to compare means between samples within each experiment.

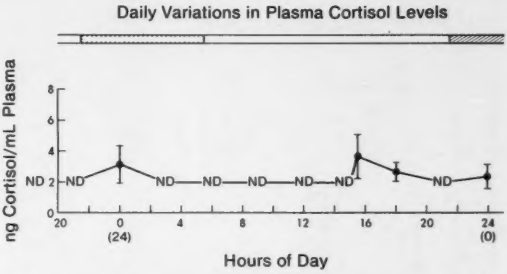

FIG. 1. Daily variations in plasma cortisol levels in fingerling rainbow trout sampled directly from hatchery raceways. Shaded area at top of graph indicates dark portion of photoperiod. Values are mean ± SE ($n = 16$). ND = nondetectable level of plasma cortisol.

Results and Discussion

DAILY VARIATIONS IN PLASMA CORTISOL LEVELS

The plasma cortisol levels of the hatchery acclimated fingerlings (Fig. 1) were generally not detectable, although at a few sample times (15:30 and 24:00) some individuals had levels (highest 16 ng/mL, lowest 2.1 ng/mL) such that the mean for the group was above the minimum level of sensitivity of the RIA. In the laboratory-acclimated fingerlings, plasma cortisol levels remained below the level of detection throughout the sampling period (data not shown); it is evident that the removal of cohorts had no effect on plasma cortisol levels of remaining fish. The results from the two experiments do not indicate the presence of a significant daily cycle in resting plasma cortisol levels in fingerling rainbow trout. Similarly, no diurnal rhythms in circulating cortisol have been previously noted for other salmonids (Strange et al. 1977). Certain nonsalmonids such as goldfish (Fryer 1975; Singley and Chavin 1975; Peter et al. 1978), carp (Redgate 1974), and gulf killifish (Srivastava and Meier 1972; Garcia and Meier 1973; Meier and Srivastava 1975) display strong diurnal variations in plasma cortisol levels, while others such as channel catfish (Boehlke et al. 1966) do not. The existence of a strong daily cycle of plasma cortisol is apparently not universal with all fishes.

The resting plasma cortisol concentrations found in the fingerlings were lower than have been described for larger rainbow trout (Hane and Robertson 1959; Donaldson and McBride 1967; Hill and Fromm 1968;

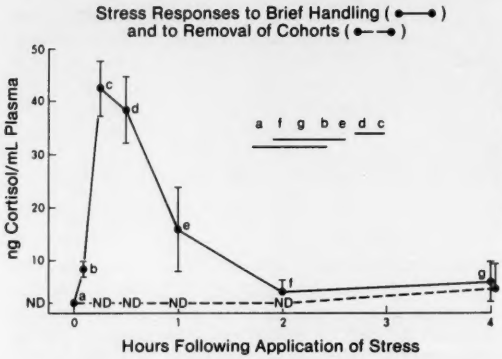

FIG. 2. Changes in plasma cortisol of fingerling rainbow trout in response to 90 s of handling and confinement, and to sampling of cohorts from the same tank. Values are mean ± SE ($n = 8$). Sample means (indicated by letter) are ranked left to right from lowest to highest. Means not significantly different (Duncan's multiple-range test, $P < 0.05$) from each other are underlined.

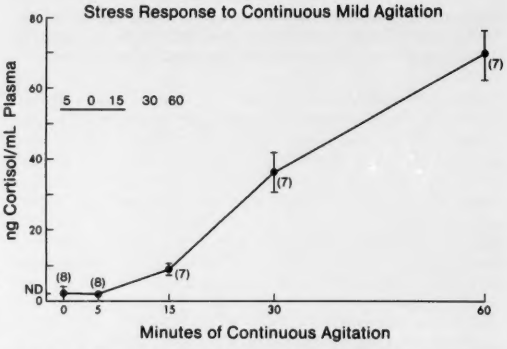

FIG. 3. Changes in plasma cortisol levels of fingerling rainbow trout subjected to continuous mild agitation and repeated capture–release within the aquarium. Values are mean ± SE. Sample sizes are in parentheses beside each value. Sample means (indicated by sample time) are ranked left to right from lowest to highest. Means not significantly different (Duncan's multiple-range test, $P < 0.05$) from each other are underlined.

Wedemeyer 1970; Grant and Mehrle 1973; R. Peter and C. Paulencu, 1978, unpublished results). Strange et al. (1977, 1978) have reported similar low basal levels of cortisol ($\leq$10 ng/mL) in juvenile chinook salmon. Hane and Robertson (1959) found that immature rainbow trout had lower levels of plasma corticosteroids (including cortisol) than mature males. Whether this relates to the relatively constant environmental conditions in the hatchery, or to size, chronological age, or stage of sexual development is not known. Lower resting levels of plasma cortisol may have also resulted from the use of an RIA which has a higher specificity for plasma cortisol than the more widely employed competitive protein-binding method (Farmer and Pierce 1974). In work on the gulf killifish, Meier and Srivastava (1975) obtained slightly lower cortisol values with RIA when using both techniques.

Brief Handling, and Removal of Cohorts

No change in plasma cortisol levels in remaining fish resulted from serial removal of cohorts from the same aquarium (Fig. 2); the mild disturbance caused by quickly removing cohorts with a dip net was not sufficient to evoke a stress response. However, plasma cortisol levels increased rapidly to a peak within 15 min after exposure to a 90-s handling and confinement stress, and then returned to resting levels by 2 h. A rapid rise in plasma cortisol followed by return to basal levels within 2 h was also reported by Strange and Schreck (1978) when chinook salmon were transferred between aquaria. From these results it is clear that mild disturbances are stressful to fingerling rainbow trout, but the response to such a stressor is transitory.

Continuous Mild Handling

An increase in the plasma cortisol level was apparent after 30 min and was increased further at 60 min during continuous mild agitation and handling (Fig. 3). Others have reported similar findings from application of mild or moderate disturbances. Wedemeyer (1969) found an increase in plasma cortisol of coho salmon 60 min after a mild handling stress similar to the stressor used in our experiment. Sockeye salmon exhibited no increase in cortisol concentration after 4 min of mild restraint, but did after 15 min of gentle agitation (Fagerlund 1967). Fryer (1975) did not observe an increase in goldfish plasma cortisol until 15 min after exposure to a handling disturbance, and Spieler (1974) found that goldfish subjected to a brief, mild restraint did not exhibit increased cortisol levels until after 10 min. Conversely, Strange et al. (1977) found that the plasma cortisol response to a handling stressor was rapid in chinook salmon, increasing at 5 ng/mL per minute, for the initial 20 min. Both presented and published results suggest that, for a particular species, the timing and magnitude of the corticoid response are dependent upon both the severity and duration of the nonspecific stressor.

Continuous Intense Handling and Severe Confinement to TLm

Plasma cortisol increased rapidly in the first 3 h of severe confinement and remained at an elevated level for the duration of the experiment (Fig. 4). A further rise in plasma cortisol was evident at 16.5 h, the approximate TLm. Some fish were moribund at 2 h and mortalities first occurred at 3.5 h. Mortality increased

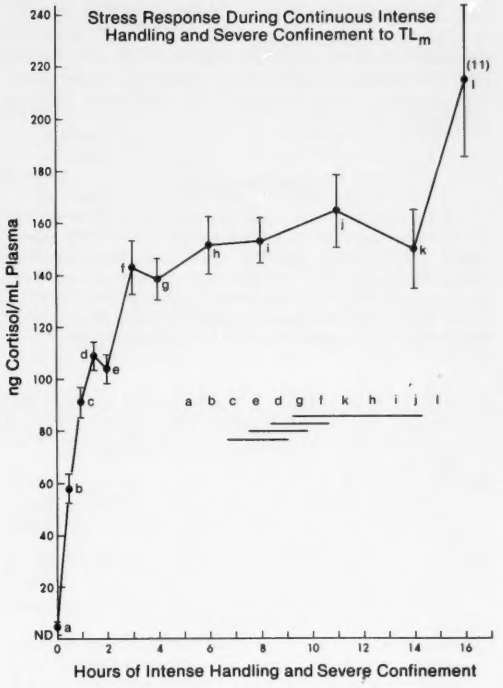
Stress Response During Continuous Intense Handling and Severe Confinement to TLm
ng Cortisol/mL Plasma
Hours of Intense Handling and Severe Confinement
a b c e d g f k h i j l
(11)

FIG. 4. Elevation in plasma cortisol of fingerling rainbow trout subjected to intense handling and severe confinement to TLm (about 16.5 h). Values are mean ± SE. Sample sizes less than 12 are in parentheses beside the mean. Sample means (indicated by letter) are ranked left to right from lowest to highest. Means not significantly different (Duncan's multiple-range test, $P < 0.05$) from each other are underlined.

progressively to the termination of the experiment at 16.5 h, at which time 56% of the remaining fish in the cage were dead. There was a strong positive correlation ($r = 0.94$) between time of death and mean weight of dead fish; the smallest fish died first. At TLm, most of the remaining fish were moribund and blood samples were difficult to obtain.

In a comparable experiment using juvenile chinook salmon, Strange et al. (1978) reported the same plasma cortisol response to severe confinement with an approximate TLm of 12.5 h. Mortality associated with this type of stressor is possibly from exhaustion of metabolic reserves and buildup of metabolic wastes, such as lactic acid. Our high correlation between size and mortality of fingerlings supports this view, as smaller hatchery-reared trout have been found to have lower liver glycogen levels (Hochachka 1961) and recover muscle glycogen more slowly after disturbance (Hochachka and Sinclair 1962) than larger fish.

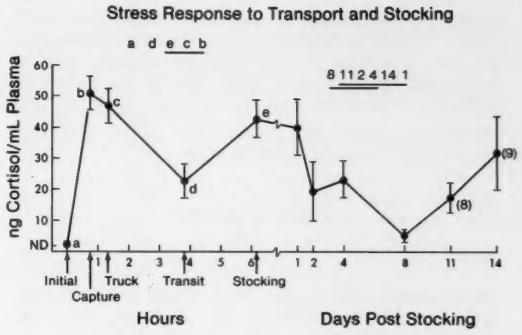

FIG. 5. Changes in plasma cortisol of fingerling rainbow trout in response to handling, transport, and stocking procedures. Values are mean ± SE for sample sizes of 12, unless otherwise indicated. Sample means (indicated by letter for stocking operation segment and by sample day for poststocking segment of experiment) are ranked left to right from lowest to highest. Means not significantly different (Duncan's multiple-range test, $P < 0.05$) from each other are underlined.

TRANSPORT AND STOCKING

During "capture" in the hatchery, plasma cortisol levels in the fingerling trout rose to the highest level observed in the experiment in 35–55 min (Fig. 5); results from 35 and 55 min during capture were not significantly different, and were pooled and expressed at 45 min on the graph. Cortisol levels were still elevated after the fish were loaded into the "truck," but decreased after 2.5 h in "transit." Plasma cortisol levels increased again during "stocking," and remained high in caged fish at 1 d after stocking. Cortisol was reduced in caged fish at 2 d and reached a significantly lower level at 8 d, but increased again through 11–14 d (Fig. 5). On these two latter dates, there were one fish and four fish dead in each cage, respectively. In each of the live cages, some fish were becoming progressively emaciated from 8 d on, and had obvious signs of abrasions ventrally, presumably from rubbing on the bottom of the cages. In addition, much of the cage mesh was clogged with algae by 14 d and food was probably in shortage.

These results indicate that the most traumatic event of the stocking operation was "capture" at the hatchery, evoking the highest stress response. Acclimation to this initial stressor after a few hours in "transit" was evident by the significantly reduced cortisol titer. However, the brief disturbance experienced at the time of "stocking" was sufficient to elevate plasma cortisol back to the initially stressed level.

The poststocking data suggest that from 4 to 8 d was required for the trout stocked into live cages to recover to an unstressed condition. These results support Strange et al. (1978), who found that the acclimation

period to a moderate confinement stressor was about 1 wk. In our experiment, the observed increase in plasma cortisol on 11 and 14 d are considered to be artifactual, as they were likely due to the combined factors of insufficient oxygen or food shortage from inadequate circulation through the cages, and skin abrasions from contact with the cage.

General Discussion

Hatchery-reared rainbow trout fingerlings, acclimated to hatchery or laboratory conditions, had low plasma cortisol levels ($\leq$2 ng/mL) and had no apparent daily cycle in plasma cortisol levels. However, fingerling rainbow trout had transitory elevations in plasma cortisol following brief mild handling, and a prolonged gradual increase in plasma cortisol with continuous mild disturbances. These results indicate that the magnitude of the increases in plasma cortisol and the time course of the changes are apparently dependent on the duration and application rate of the mild stressor. The severity of the stressor is obviously important in the magnitude of the increase in plasma cortisol levels; with the onset of continuous intense handling and severe confinement, there was a very rapid increase in plasma cortisol to a plateau two to four times the levels found as a result of the mild stressors. Furthermore, the high levels of plasma cortisol were maintained during the continuous application of the severe stressor up to and including the time when the fish became moribund and died. The plasma cortisol levels observed in these experiments provide a useful index for comparison with the levels found during a stocking operation.

Many small, shallow prairie lakes in Canada are stocked with rainbow trout for recreational fishing (Miller and Thomas 1956) or for fish farming (Lawler et al. 1974). When stocked into such lakes, trout are subjected to a variety of secondary stressors, for example, adverse water chemistry, sudden temperature changes, and predators. These have been implicated as causes of high mortality (Ayles et al. 1976; Myers and Peterka 1976). Our results indicate that the fish arrive at the lake in an already stressed condition as a result of the normal delivery operation. However, in our experiments, the plasma cortisol levels found during the stocking operation and poststocking in the lake were similar to the levels found in fingerlings exposed to mild stressors. Since the stocking operation does not represent a severe stressor to the fish, what is likely of significance is that the fish are continuously stressed for a prolonged period even after planting in the lake. Under these circumstances, high delayed mortality may result either from exhaustion or directly through action by some secondary stressor in the new environment. The poststocking stress recovery period (= acclimation period?) of approximately a week demonstrated in this experiment and by Strange et al. (1978) may be important to poststocking survival. If so, this would help to explain the significantly higher returns of rainbow trout kept in holding cages over those stocked directly, as documented by Ayles et al. (1976). By reducing the initial stocking stress and determining what factors may shorten the stress recovery period following planting, mortality may be significantly reduced in stocking operations.

Acknowledgments

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Study of *Haematractidium scombri* in Atlantic Mackerel, *Scomber scombrus*

SHARON A. MACLEAN

Northeast Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Oxford, MD 21654, USA

MACLEAN, S. A. 1980. Study of *Haematractidium scombri* in Atlantic mackerel, *Scomber scombrus*. Can. J. Fish. Aquat. Sci. 37: 812–816.

The prevalence of *Haematractidium scombri*, an intraerythrocytic protozoan, was studied over a 3-yr period in Atlantic mackerel migrating off Chincoteague, Virginia. In 1974, 24%, in 1975, 42%, and in 1976, 45.3% of the fish examined were infected. Age-2 mackerel (measuring 25–28 cm fork length) were more frequently and more heavily infected than older fish. Adult mackerel from Boothbay Harbor, Maine, and from the bottom overwintering population in the Gulf of Maine also were infected; however, *H. scombri* was not found in blood smears of age-0 mackerel from Montauk, Long Island, New York. *Haematractidium* was not found in tissue sections, but structures that might be exoerythrocytic stages were seen in kidney and spleen imprints.

Key words: Haematractidium, protozoan, mackerel, *Scomber*

MACLEAN, S. A. 1980. Study of *Haematractidium scombri* in Atlantic mackerel, *Scomber scombrus*. Can. J. Fish. Aquat. Sci. 37: 812–816.

L'auteur a étudié sur une période de 3 ans l'occurrence de *Haematractidium scombri*, un protozoaire intra-érythrocytaire, chez le maquereau bleu migrateur, au large de Chincoteague, en Virginie. En 1974, 24%, en 1975, 42% et en 1976, 45,3% des poissons examinés étaient infectés. Les maquereaux d'âge 2 (mesurant 25–28 cm de longueur à la fourche) étaient plus fréquemment et plus sévèrement infectés que les sujets plus âgés. Les maquereaux adultes provenant de Boothbay Harbour, dans le Maine, et de la population passant l'hiver en profondeur dans le golfe du Maine étaient aussi infectés; cependant, on n'a pas trouvé de *H. scombri* dans des frottis de sang de maquereaux d'âge 0 de Montauk, Long Island, New York. *Haematractidium* n'a pas été trouvé dans des coupes tissulaires, mais des structures pouvant être des stades exoérythrocytaires ont été observées dans des impressions de rein et de rate.

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HENRY (1910) was one of the first to report intraerythrocytic parasites in marine fish other than the commonly observed hemogregarines. One of these parasites was *Haematractidium scombri* found in mackerel from the Irish Sea (Henry 1913a, b). Henry's recognition and suggested classification of *H. scombri* had been questioned (Wenyon 1965) but more recent ultrastructural studies by Johnston (1975) support Henry and suggest that *H. scombri* has certain affinities with the piroplasms, which are considered to be Apicomplexa protozoans (Levine 1971).

Haematractidium has not been the subject of much study. Besides the preceding references, there is only one additional report, by Newborg and Miller (1975), of *H. scombri* found in mackerel of the Northwest Atlantic. In general, little attention has been given to the interactions between parasites and wild fish populations; and, in particular, no information existed on *H. scombri* in the Atlantic mackerel. Therefore, the present study was conducted to establish the prevalence of *H. scombri* in the North American population of mackerel and to provide qualitative and quantitative data on the host–parasite relationship in the survey area off Chincoteague, Virginia. Additional information is provided on *H. scombri* infection in mackerel from more northern locations to establish the condition and persistence of infection during the host's seasonal migration.

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Materials and Methods

Data are based on samples taken from 200 adult mackerel collected 8–16 km off Chincoteague, Virginia, by trawl or hook-and-line in the springs of 1974 through 1976. Duplicate smears of blood taken from the branchial artery were made from all the fish. Samples of spleen, liver, head kidney, and intestine were taken from 36 fish in 1975, and kidney and spleen imprints were made from 36 fish in 1976. Observations of gross abnormalities (ectoparasites, pallor, skin lesions, fin rot, etc.) were recorded. Weight and length measurements were recorded for each specimen.

In addition to the above survey samples, blood smears

TABLE 1. Sampling dates, location, and methods of *Scomber scombrus* and prevalence of *Haematractidium scombri*.

Location	Date	Capture method	No. fish	Mean forklength (cm) (range)	% infection by *H. scombri*
Chincoteague	April 1974	Trawl	100	28.9 (22.1–36.1)	25
	April 1975	Trawl	36[a]	28.4 (23.9–32.8)	42
	March 1976	Hook-and-line	64[b]	30.2 (23.1–37.6)	45
Montauk	Aug. 1975	Hook-and-line	14[a]	18.8 (17.8–19.8)	0
Boothbay Harbor	Oct. 1973	Hook-and-line	93	— (27.9–36.8)	32.3
	July, Aug. 1976	Hook-and-line	30	— (27.9–36.8)	46.7
	Sept. 1976	Hook-and-line	25	— (27.9–36.8)	56.0
Gulf of Maine	Feb. 1978	Trawl	31	34.5 (32.0–39.0)	19.4
	June 1978	Trawl	25	35.6 (28.0–39.0)	28.0

[a]Liver, spleen, head kidney, and intestine sampled from all.
[b]Head kidney and spleen imprints made from 36 fish.

were made and tissues sampled from 14 age-0 mackerel collected by hook-and-line in 1975 from Montauk, Long Island, New York. Giemsa-stained blood smears of 148 adult mackerel received from Mr Stuart Sherburne, State of Maine, Department of Sea and Shore Fisheries, also were examined. These fish had been captured by hook-and-line along the shore in Boothbay Harbor, Maine, in the fall of 1973, and summer and fall of 1976. Unstained blood smears of 56 adult mackerel collected in the Gulf of Maine were received for examination. These fish had been taken by trawl during routine groundfish surveys conducted by the Northeast Fisheries Center, National Marine Fisheries Service, in February and June 1978.

Table 1 summarizes the sampling dates, locations, methods, and size of fish.

Blood smears and tissue imprints were fixed in 100% methanol for 5 min, air-dried, and stained with Wright's blood stain. Tissues were excised on shipboard, fixed in Davidson's fixative, and later processed histologically. Tissue sections were cut at 6 μm and stained with hematoxylin–eosin, Mallory's trichrome, or treated by the Feulgen reaction. Photographs were taken with a Zeiss photomicroscope.

Each blood smear was examined for 15 to 25 min with an oil-immersion lens. Parasitemias were designated as light, moderate, or heavy based on the number of parasites observed per 10 000 red blood cells (RBCs). Light infections had 1–19 (mean 14.4) parasites/10 000 RBCs; medium infections had 24–58 (mean 34.6)/10 000 RBCs; heavy infections had 72–532 (mean 98.0)/10 000 RBCs.

Results

Haematractidium scombri in the erythrocytes of *Scomber scombrus* had mean length and width measurements of 6.4 and 3.4 μm, respectively. With Wright's or Giemsa's stain, the parasite was uniformly very pale blue, lacked a sharp outline, and usually had a faint nonstaining area near its nucleus. The central nucleus was small ($<1\ \mu$m) in contrast to that of hemogregarines common in marine fishes. Often, the parasite lay against the host cell nucleus, seeming almost to adhere to it. Frequently, cytoplasmic extensions of the protozoan partially surrounded the erythrocyte nucleus (Fig. 1).

Haematractidium was frequently associated with lysing erythrocytes and remained in close association with the nucleus of the lysing cells (Fig. 2). Quite often, the parasite was observed extracellularly, usually in smears from the heaviest infections. Extracellular forms were always adjacent to an indistinct pink-staining object, presumably the remains of the host cell nucleus. One, two, and occasionally three parasites were observed within a single erythrocyte. Forms resembling malarial signet rings (Fig. 3) were common in blood smears of mackerel from Boothbay Harbor but were infrequent in blood smears of other samples.

CHINCOTEAGUE SURVEY

Table 2 presents the combined data for the 3-yr survey of Chincoteague mackerel and demonstrates the relationship between fish length and infection by *H. scombri*. Age-2 mackerel were the principal hosts of *H. scombri* and had 56.3% (36/64) infection as compared to 27.8% (5/18) for age-1, and 29.6% (24/81) for age-3 fish. Mackerel age-4 or over had 10.8% (4/37) infection. Furthermore, 16 of the total 25

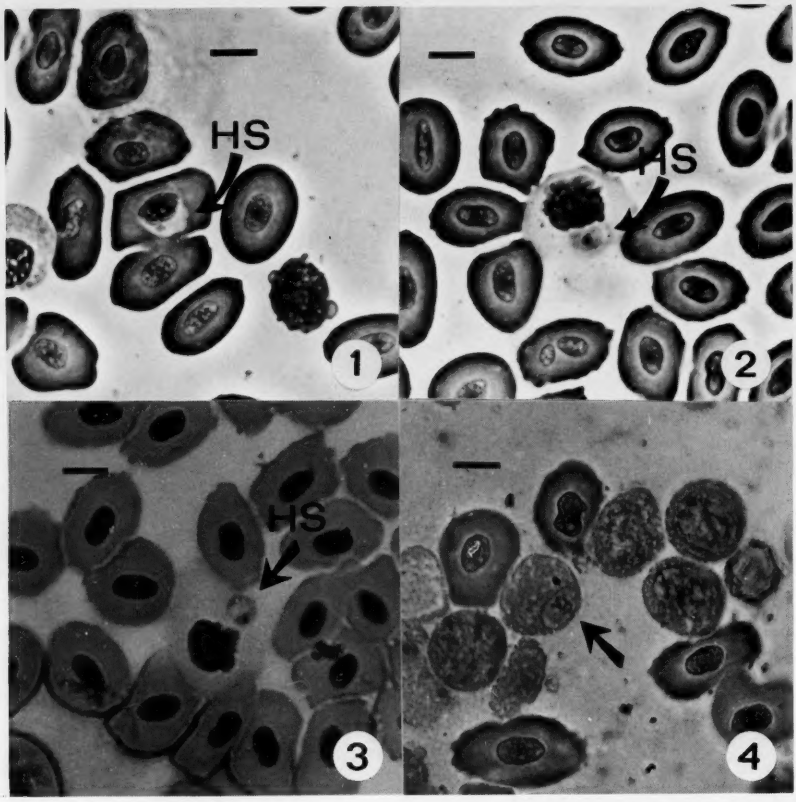

FIG. 1–3. *Haematractidium scombri* (HS) in blood smears of *Scomber scombrus*. (1) Intraerythrocytic form. Note adherence to the host cell nucleus. Wright's stain. Phase contrast. (2) In a lysed erythrocyte. Wright's stain. Phase contrast. (3) Ring form in a lysed erythrocyte. Wright's stain. In all figures, bar = 5 μm. FIG. 4. Atypical leucocyte in a kidney imprint (*H. scombri??*). Wright's stain. Bar = 5 μm.

(64.0%) heavy infections were in age-2 fish.

Haematractidium scombri was not seen in the sections of liver, kidney, spleen, and intestine of 36 mackerel collected at Chincoteague in 1975, although other parasites were present. Cysts of the coccidian, *Eimeria clupearum*, were found in the livers of 95% (34/36) of the mackerel, and possible macrogametocytes of this parasite were in the intestine.

Haematractidium scombri was found in erythrocytes in kidney and spleen imprints of mackerel sampled in 1976 that had patent infections. Lysing erythrocytes found in imprint preparations frequently were associated with ring forms and occasionally with multiple *H. scombri*. Ring forms were most common in imprints of fish that were negative on the basis of blood smear examination. Large, lightly or nonstaining bodies with large, diffuse, central structures were found in the cytoplasm of unidentified leucocytes located primarily in the spleen and also in the kidney tissue (Fig. 4). The nucleus of the leucocyte was indented by the presence of this body. Occurrence of these atypical leucocytes did not correlate with the presence of *H. scombri* in circulating erythrocytes.

TABLE 2. *Haematractidium scombri* infection according to age[a] in 200 mackerel from Chincoteague, Virginia, 1974–76. Combined data.

	Age-1 21–24 cm	Age-2 25–28 cm	Age-3 29–32 cm	≥Age-4 33–37 cm
No. examined	18	64	81	37
No. (%) infected	5 (27.8)	36 (56.3)	24 (29.6)	4 (10.8)
No heavy infections	3	6	4	0
% (no.) of total heavy infections	12.0 (3/25)	64.0 (16/25)	24.0 (6/25)	0 (0/25)

[a]Age–length correlation from Pentilla and Anderson 1976 otolith-determined age–length keys.

Northern Collections

Haematractidium was found in each of the sample lots received from Boothbay Harbor and the Gulf of Maine (Table 1). Light infections were the most common. Thirty (51.8%) of the 58 infections found in Boothbay Harbor mackerel were light infections and only 14 (24.1%) were heavy. Twelve (92.3%) of the 13 infections found in 56 mackerel from the Gulf of Maine were light infections. As Table 1 indicates, the fish from these two locations were generally larger than 28 cm fork length. Intracellular ring forms of *H. scombri* and the presence of two and sometimes three parasites per erythrocyte were common in the Boothbay samples.

Fourteen age-0 mackerel sampled from Montauk, Long Island, were not parasitized by *H. scombri* in the circulating blood cells or kidney and spleen tissues. *Eimeria clupearum*, however, was found in the liver of 1 of the 14 fish.

Discussion

Prevalence of *Haematractidium* infection nearly doubled during the 3-yr sampling period at Chincoteague. In 1974, 25 of 100 (25%) mackerel were infected while in 1976, 29 of 64 (45%) were infected. Different collection methods were used, but major schools of migrating mackerel were sampled during these collections; fish were selected randomly on both occasions, and the mean size of the specimens essentially was the same (Table 1). Therefore, these percentages are considered to be valid indicators of a marked change in the prevalance of *H. scombri* in mackerel off Chincoteague. (The 1975 collection at Chincoteague is not included in this discussion because specimens were selected on the basis of size to collect infected fish for tissue examinations.) The data also suggested an increase in infection rate over the years in inshore mackerel from Boothbay Harbor, Maine. In October 1973, 32.3% of the mackerel sampled were infected whereas 46–56% were infected in 1976.

Based on the Chincoteague survey, it is apparent that the principal hosts of *Haematractidium* are age-2 mackerel and that these fish have a greater percentage of heavy infections than older fish. Possibly, *H. scombri* infects and kills its younger hosts. However, infected fish were not obviously weaker or different or apparently more stressed during sampling than uninfected fish, and no external abnormalities (pallor, lesions, etc.) were associated with infected fish. The long-term persistence of this parasite in the European population of mackerel is obvious from the reports by Henry (1910) and Johnston (1975). This indicates a well-established host–parasite relationship. It is suggested by the present short-term study that this relationship exists in the North American population of mackerel also. The decreased prevalence and intensity of infection in age-3 and older mackerel may be due to elimination of heavily infected younger fish from the population, or due to an immune response by the host. Age-1 mackerel also had a lesser prevalence and intensity of infection than age-2 fish. This variance in infection may be due to differences in distribution and migratory habits of age-1 and age-2 fish since age-1 mackerel begin migration a month later than, and on a course parallel to, that of older mackerel (Sette 1950). The mode of transmission and location of fish at time of infection are as yet unknown, and differences in distribution and habits of age-1 and age-2 mackerel may be significant factors influencing the exposure of fish to infection.

Haematractidium was associated primarily with lysing erythrocytes or their remains. However, lysing erythrocytes were frequently abundant in blood smears of fish not infected with *H. scombri*. Henry (1913b) also found lysing erythrocytes in *H. scombri* infections, but Wenyon (1965) considered this to be a result of mechanical damage during spreading of the blood. The frequency with which I found *H. scombri* associated with lysed cells suggests that *Haematractidium* may increase the fragility of the erythrocytes, although obviously other factors affect the cells in a similar manner.

Heavy infections of *H. scombri* averaged less than 100 parasites per 10 000 (1%) erythrocytes. Destruction of so few blood cells, and even of cells in the heaviest infection (532/10 000 = 5.3%), probably would not stress the host significantly. Low level parasitemias are typical of intraerythrocytic protozoans of marine fishes (Laird and Bullock 1969; Saunders 1960; So 1972). There is little information concerning the pathologic effects of hematozoan parasites on fish and there are no reports implicating intraerythrocytic parasites as causative agents in mortalities in wild populations of fish. Traditional hematological techniques were not practical under the field conditions of this study but would be useful in determining an effect, if any, on the host.

The life cycle of *H. scombri* is unknown; therefore, blood smears of age-0 mackerel from Montauk, Long Island, were examined in attempts to find early stages of infection. Present evidence indicates that age-0 mackerel are not parasitized by *H. scombri*, yet age-1 mackerel migrating off Chincoteague are infected (Table 2). According to Sette (1943), age-0 mackerel are found close to shore in summer and do not migrate until they move offshore in late fall. After overwintering on the bottom, they appear in coastal waters the following spring as age-1 mackerel. Perhaps infection occurs during the spring oceanic migration or during the overwintering period when mackerel are found on the edge of the continental shelf.

Johnston (1975) reported that ring forms of *H. scombri* were common in the circulating blood of the European population of mackerel sampled in September. In the present study, ring forms were rarely seen in blood smears of Chincoteague mackerel captured in spring but were present in erythrocytes of the tissue imprints. In contrast, mackerel sampled from Boothbay Harbor, Maine, in the summer and fall often had ring

forms in the circulating blood. The significance of ring forms and relationship of patent infections of these to the latter part of the migratory phase of the host (summer and fall vs. spring) is unclear. Ring forms may represent different stages, either early or senescent, in the development of the parasite and should be examined more closely.

No known reproductive stages of protozoans were observed in mackerel erythrocytes; the site and form of reproduction of *H. scombri* are still unknown. Henry (1913b) illustrated stages of *H. scombri* found in the spleen of mackerel which closely resemble the bodies I saw in the atypical leucocytes in kidney and spleen imprints. Interestingly, the schizonts described and illustrated by Laird (1953) of *Hemogregarina bigemina* in leucocytes of marine fishes also have similarities to these bodies in mackerel leucocytes, i.e. lightly stained cytoplasm, diffuse nucleus, and indented host cell nucleus. The affinities *H. scombri* has with piroplasms (Johnston 1975) and hemogregarines (Laird 1953) complicate the classification of this parasite. The bodies found in atypical leucocytes may be a stage of *Haematractidium* but experimental studies would be necessary to demonstrate their place in the developmental cycle and to classify the organism properly. Since mackerel and herring of the Western Atlantic share some parasites (Lubieniecki 1973; Pellérdy 1965; Sindermann 1958), a detailed study of the blood protozoa of herring may show that this species is infected with *H. scombri* and possibly disclose some useful life cycle information.

Sette (1950) reported distinct northern and southern stocks of mackerel in the Northwest Atlantic having different migratory routes which temporarily overlap south of New England. Traditional fish-tagging studies have suggested further mixing of these two stocks during the overwintering period (Parsons and Moores 1974) when mackerel are found near the bottom at the edge of the continental shelf. However, the degree of this mixing has not been determined adequately. As a supplement to traditional tagging studies, the application of *H. scombri* as a natural biological tag may prove useful in determining the extent of mixing of the two stocks. Sette's (1950) hypothesis that the southern stock of mackerel migrates into the Gulf of Maine is supported by the similarity of *H. scombri* prevalence in fish captured in 1976 off Chincoteague in spring and Boothbay Harbor in summer (see Table 1). The strictly northern stock of mackerel, presumably in the Gulf of St. Lawrence, was not sampled in this study. The intermixing of the two stocks may render them homogeneous for *H. scombri* prevalence. However, the homogeneity or heterogeneity of the parasite's prevalence in the two separate stocks should be given consideration in the future.

Acknowledgments

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Measurement of Whole Lake Sediment Accumulation and Phosphorus Retention Using Lead-210 Dating

R. Douglas Evans and Frank H. Rigler

McGill University, Department of Biology, Montreal (Qué.) H3A 1B1

Evans, R. D., and F. H. Rigler. 1980. Measurement of whole lake sediment accumulation and phosphorus retention using lead-210 dating. Can. J. Fish. Aquat. Sci. 37: 817–822.

Lead-210 dating was used to measure rates of sediment accumulation in 15 cores from Bob Lake, Ontario. The rate of accumulation was highly correlated with sample depth. This relation allowed the calculation of accumulation of sediments over the whole lake area. Phosphorus (P) retention was calculated from mean concentration of P in the sediments and the whole lake accumulation of sediment. Retention of P calculated in this way was similar to retention calculated from previously measured input and output of P.

Key words: lead-210 dating, sediment accumulation, phosphorus retention

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Les taux d'accumulation des sédiments dans 15 carottes du lac Bob, en Ontario, ont été mesurés par datation au plomb-210. Il y a étroite corrélation entre taux d'accumulation et profondeur de l'échantillon. Cette relation permet de calculer l'accumulation des sédiments à la grandeur du lac. Nous avons calculé la rétention du phosphore (P) d'après la concentration moyenne de P dans les sédiments et l'accumulation du sédiment dans le lac entier. La rétention de P calculée de cette manière est identique à celle calculée selon mesures antérieures d'entrées-sorties de P.

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Knowledge of the net accumulation of materials in the sediments of a lake is potentially useful for modeling nutrient concentrations in water (Vollenweider 1969; Dillon and Rigler 1974), for establishing the rate of return from the sediments for some substance for which influx is known (Schindler et al. 1976), and for testing the validity of energy flow data (Rigler 1978). This information would be equally useful outside the field of limnology as, for example, to geomorphologists concerned with regional erosion rates.

Despite their obvious value, data on the net accumulation rate of sediments for a whole lake basin have rarely been gathered (Wilson 1936; de March 1978; Kimmel 1978), presumably because of the difficulty of such measurements and uncertainty about their validity.

Problems arise from two sources. First, valid results depend on a reliable method of measuring the rate at which sediments are accumulating at a particular location. Second, sediments accumulate at different rates at different parts of a lake (de March 1978; Kimmel 1978). Consequently many samples must be taken at various sites in the lake if a good estimate of average sedimentation is to be obtained. Our research was addressed to these two difficulties.

Several studies have suggested that depth in the lake may be the most important factor governing spatial variation in sediment accumulation rates (Wilson 1936; Wieckowski 1969; Davis et al. 1971; Lehman 1975). If this were true, a random sampling pattern, stratified with respect to depth, should give a good estimate of average sediment accumulation. However, two studies have suggested that factors other than depth can have an important influence. de March (1978) examined sediment accumulation in Char Lake, a polar lake with dense moss beds at intermediate depths. He found that, presumably because of trapping of sediments by the moss beds, sedimentation was higher there than at shallower or deeper sites and at sites of equal depth where moss did not grow. Kimmel (1978), studying Castle Lake, concluded that depth explained only 30% of the variation in rates of sediment accumulation. Other factors, steepness of slope being an important one, accounted for much of the remaining variability. Castle Lake is unusual in that it is highly sheltered from prevailing winds and has an exceptionally steep sloping bottom. Edgington and Robbins (1976) have shown nonmorphometric patterns of sediment deposition in Lake Michigan. They propose wind-driven currents as the mechanism for the observed deposition but given the size of Lake Michigan many more samples would be required than the few taken by Edgington and Robbins before any firm conclusions could be drawn. Our hypothesis was that in a more morphometrically typical, temperate lake, depth would be the main factor causing variation in the rate of sediment accumulation. Thus

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we chose Bob Lake in southern Ontario (44°54′N, 78°47′W); a 0.23-km^2 lake with a maximum depth of 60 m. Morphometry is shown in Fig. 1. We predicted that in this lake depth would be the most important variable influencing sediment accumulation rates.

Theoretically, the best method for measuring present accumulation is one, such as the ^{210}Pb method, which depends on decay of a short-lived isotope. Markers such as volcanic ash layers (deposited at ~3370 BP) used by Kimmel, and marine sediments (~6600 BP) used by de March (1978), or long-lived isotopes such as ^{14}C (Likens and Davis 1975) integrate sediment accumulation over too long a period to be used with confidence to measure present accumulation rate. Other possible methods for dating recent accumulation include ^{232}Th/^{228}Th ratios (Koide et al. 1973) and low frequency echo sounding (d'Anglejan and Brisebois 1974; King 1966). Even the interpretation of ^{210}Pb results has been questioned (Robbins 1978; Appleby and Oldfield 1978). Controversy arises from the generally made assumption of constant rate of accumulation of the sediments. Where varved sediments are produced, as in meromictic lakes, ^{210}Pb dating and varve counts can be compared to establish the validity of the accumulation rate predicted by the ^{210}Pb dating (Koide et al. 1972; Appleby et al. 1979). In general there is good agreement between accumulation rates calculated by the two methods. Examination of the flux of ^{210}Pb to the varved sediments of Fayetteville Green Lake reveals year to year variation of less than 25% (G. J. Brunskill, Department of Fisheries and Oceans, Winnipeg, 1979, personal communication) in keeping with the assumption of constant flux of ^{210}Pb to the sediments. In dimictic lakes these tests of the model are not feasible. Making the assumption that the method gives valid results in dimictic lakes, we have chosen to test the applicability of ^{210}Pb dating to the measurement of whole lake accumulation by applying it to a lake for which phosphorus budgets had been thoroughly measured. Bob Lake, previously studied by Dillon and Rigler (1974) and Cross (1977), was suitable on this count also. From their studies we could obtain an estimate of annual accumulation of P in the sediments. This estimate could then be compared with an independent estimate derived from ^{210}Pb dating of the sediments and their P content.

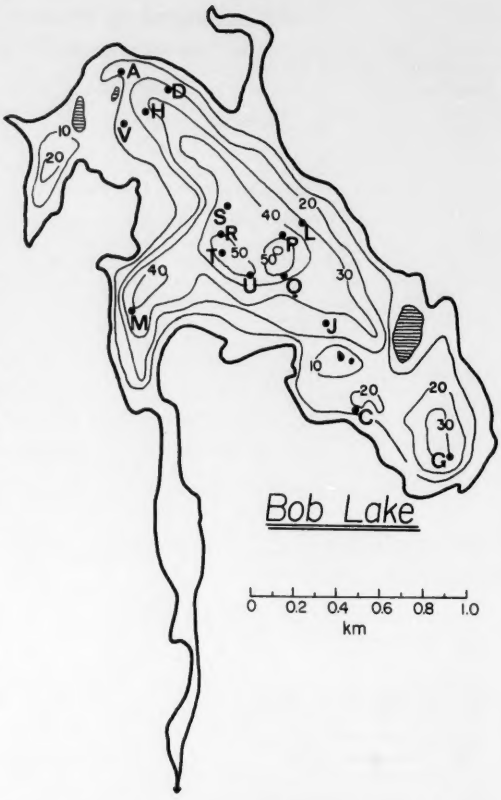

FIG. 1. Morphometry and sampling locations, depth contours are in tens of metres.

Methods

The coring sites in Bob Lake are shown in Fig. 1. Sediment cores were taken with a K–B corer (Brinkhurst et al. 1969) in 1975 and 1976 by Cross (1977). Cores were sectioned into 2-cm slices within 24 h of being collected, sealed in polycarbonate vials, and frozen until analyzed for ^{210}Pb and P. Of the 50 cores collected by Cross, 15 were selected to represent most areas and depths of the lake. None was taken at depths less than 10 m because the bottom materials there were too hard to be sampled by the K–B corer.

The sediment samples were massed, dried at 105°C for 24 h, and remassed. Initially ^{210}Pb was measured by the method described by Koide et al. (1972). In this method the beta-emitting daughter (bismuth-210) of ^{210}Pb in secular equilibrium is assayed. This method, although satisfactory, was too slow. Consequently, in most cores, we assayed ^{210}Po, the granddaughter of ^{210}Pb. The ^{210}Po was extracted from the dry sediments by the volatilization technique of Eakins and Morrison (1978) and deposited on silver discs according to Flynn (1968). All ^{210}Po samples were assayed in a Canberra alpha spectrometer equipped with Princeton Gamma Tech surface-barrier detectors (active area 300 mm^2). The isotope ^{208}Po, produced in the McGill University cyclotron by irradiation of bismuth foil, was added to the dry sediments as a yield indicator to measure the efficiency with which ^{210}Po was extracted and plated. The absolute activity of the ^{208}Po was calibrated against a ^{210}Po source supplied by Amersham Corporation. In general recovery of the ^{208}Po was above 60%.

Total phosphorus in the sediments was measured by the method of Anderson (1976). Dry sediment was ashed at 550°C for 6 h, and the residue was massed and dissolved in dilute HCl. A subsample was then diluted to 50 mL and

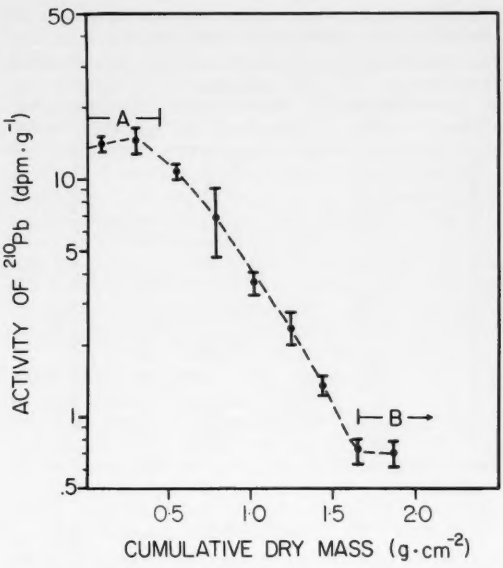

FIG. 2. A typical ^{210}Pb profile. Data are for core L. Error bars are 1σ counting errors.

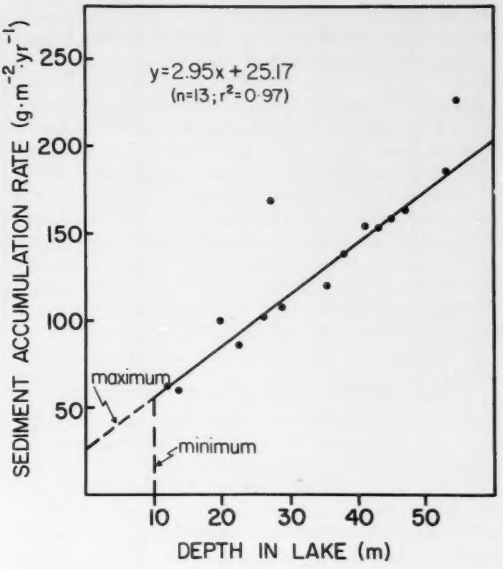

FIG. 3. The relation between sediment accumulation rate and lake depth.

phosphorus in it determined colorimetrically (Strickland and Parsons 1968).

CALCULATION OF ACCUMULATION RATES FROM LEAD-210 ACTIVITY

Since there has been some dispute as to the proper method of interpreting ^{210}Pb profiles in sediments (Robbins 1978; Appleby and Oldfield 1978), we will describe our method and the assumptions involved in it. We assume that over the last century the sediments in Bob Lake have accumulated at a constant rate. We assume that profiles deviate from a simple log-linear relation near the surface (shown as A in Fig. 2) because activity of benthic organisms and water currents mix the surface sediments. Deviation from log-linearity far below the surface (B in Fig. 2) is a result of ^{210}Pb continually being produced at all depths by decay of in situ parent radium. Further we assume that the radium content of dry sediments is constant with depth. Finally, we assume that the flux of ^{210}Pb to the sediment surface has been constant through time.

Provided these assumptions are valid the ^{210}Pb content of the sediments will decrease exponentially with depth to an asymptote of the concentration C, where C is the supported ^{210}Pb level. Thus below the mixed layer, the concentration of ^{210}Pb (C_w) at depth w is described by:

$$(1)\quad C_w = Co\, e^{-kw} + C$$

where C_w is the concentration of ^{210}Pb measured in dpm·g^{-1} dry wt, w is the depth measured as g dry mass/cm^2, Co is the surface concentration ^{210}Pb, and C is the concentration of supported ^{210}Pb in the sediments. Depth is expressed as cumulative dry mass to avoid the problems caused by compaction of the sediment through time (Robbins 1978). The slope of the regression of $\ln(C_w - C)$ on w gives the constant (k) of decrease of unsupported ^{210}Pb with depths expressed as g^{-1}·cm^2, and the sediment accumulation rate (r) in g·cm^{-2}·yr^{-1} is given by:

$$(2)\quad r = \lambda/k$$

where λ is the decay constant of ^{210}Pb (0.03114 yr^{-1}).

To derive k a linear regression was calculated through the three points of highest activity in the section in which unsupported radioactivity decreased exponentially with depth. This procedure was adopted because the plot usually deviated from linearity as zero activity was approached. This deviation could merely indicate that C is consistently overestimated. By neglecting data where $C_w \simeq C$, we have minimized effects of this possible error. A similar unexplained deviation from linearity can be seen in the data of Schubel and Hirschberg (1977).

Results

Calculated rates of sediment accumulation are plotted against depth in the lake at which the cores were taken in Fig. 3. It shows a strong correlation between sediment accumulation rate and depth. It also shows that two sites are anomalous. There is nothing distinctive about the location of the two anomalous cores (G and U in Fig. 1). Although there are many possible explanations for such anomalous rates of accumulation, we cannot presently discern the actual cause of these results. Improper coring can result in sediment mixing and an overestimation of the actual

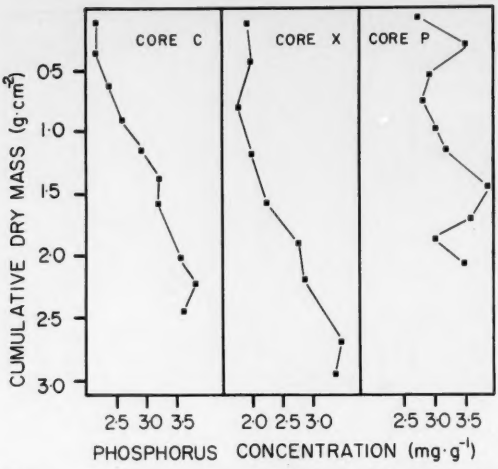

FIG. 4. Typical phosphorus profiles from three Bob Lake cores.

accumulation rate. Core U shows some evidence of corer-induced mixing as defined by Kipphut (1978). It is also possible that there are small areas where morphometry or water movement enhance deposition, although the evidence would suggest these sites occupy a very small percentage of the lake bottom. Our assumption is that cores G and U are not representative of the accumulation rates for the depths from which the samples were taken and will not be included in the following calculations.

For the remaining 13 sites the regression of the sediment accumulation in $g \cdot m^{-2} \cdot yr^{-1}(y)$ on sample depth in metres (x) is:

$$(3)\quad y = 2.95\,x + 25.17 \quad (n = 13, r^2 = 0.97).$$

Thus, depth alone explained 97% of the variation between sites in the rate of sediment accumulation. With the inclusion of sites G and U in the regression 82% of the variation is accounted for.

Three typical profiles of phosphorus concentration in Bob Lake sediments are shown in Fig. 4. The profiles vary from uniform concentration with depth to slightly increasing concentration with depth. As with the ^{210}Pb profiles depth has been calculated as a cumulative weight to eliminate the effects of compaction. We have chosen to use the surficial concentration (0–2 cm) as the one most representative of recent sedimenting material. Although processes such as diagenesis can alter concentration profiles in sediments, there is no evidence (such as a large increase towards the sediment surface) for these processes in Bob Lake. In the absence of information to the contrary we feel that the surficial section of sediment offers the best estimate of current sediment phosphorus in our lake. The P concentration in this section will be used in subsequent calculations and these data are given in Table 1.

TABLE 1. Variation in sediment accumulation rate and phosphorus concentration in 0–2-cm sediments.

Core	Lake depth (m)	Sediment accumulation rate ($g \cdot m^{-2} \cdot yr^{-1}$)	Phosphorus ($mg \cdot g^{-1}$ dry wt)
A	11.5	61.0	1.7
V	13.5	59.5	1.9
B	16.5		2.0
C	19.6	99.0	2.2
D	22.4	85.7	2.2
E	22.6		2.3
W	23.0		2.4
G	27.0	102.0	2.1
X	27.0		1.9
I	27.8	168.0	2.3
J	28.8		2.4
K	31.6		2.7
L	35.5	119.4	2.4
M	38.0	138.0	2.1
N	39.5		2.4
O	40.5		2.5
P	41.0	154.0	2.5
Q	43.0	152.0	2.6
R	45.0	156.9	2.6
Y	45.0		2.4
S	47.0	162.7	3.0
T	52.9	185.1	2.4
U	53.4	226.0	2.6

Table 1 shows that although the horizontal variation in P concentration of surface sediment is not large, there is a trend to higher P concentration at deeper depths in the lake. A linear regression of P concentration on depth showed a significant correlation. Fifty-five percent of the variation in P concentration was explained by depth. Thus we felt that a simple average of all samples was not sufficient and chose to use the regression equation:

$$(4)\quad P = 0.018\,d + 1.75 \quad (n = 23, r^2 = 0.54)$$

where P is phosphorus concentration in $mg \cdot g^{-1}$ and d is depth in metres, in the calculation of lake retention of P.

CALCULATON OF WHOLE LAKE RATES

To calculate the total accumulation of sediments in Bob Lake we must make an assumption about the sediment accumulation at water depths less than 10 m because the bottom material in this region, which represents 39% of the lake bottom, was too hard to be cored. Evidence from cores collected by divers in similar areas of lakes in the vicinity of Bob Lake suggest that this material is primarily sand and gravel and that the water content of sediments in the shallows is much lower than in sediments from the centre of the

lake (unpublished data). The best we can do for the present is to set upper and lower limits on the accumulation from 0 to 10 m and use these. The lowest limit would result from assuming that zero accumulation occurs at depths from 0 to 10 m. This would be equivalent to assuming that the K–B corer did not work in this area because no sediments were accumulating. This assumption would underestimate total accumulation. The upper limit would be given by extrapolating the regression equation (3) to zero depth (see Fig. 3). That this procedure would overestimate total accumulation is supported by the work of Kipphut (1978), and Likens and Davis (1975), which suggested that the bottom of the summer epilimnion is the depth above which no permanent sediments accumulate. This would be ~6 m in Bob Lake. The most likely situation between these extremes is that sediment accumulation decreases rapidly between 10 and 5 m.

For all depths the estimated (0–10 m) or predicted (10–60 m) mean sedimentation rate for each 5-m stratum was applied to the area of the stratum and all strata were summed to give the total sediment accumulation. Expressed per unit of lake surface area, the average sediment accumulation for Bob Lake is betweeen 63 and 78 g dry matter per m^2 per year.

Calculations were made using equation (4) to determine the mean P concentration for each stratum. The whole lake P retention was calculated by summation of the products of mean P and mean accumulation rate for each stratum. Using this technique the rate of accumulation of phosphorus in the sediments of Bob Lake falls between 143 and 170 mg·m^{-2}·yr^{-1}.

Discussion

The results of this study suggest that in medium-sized temperate lakes with a single basin, the measurement of total annual accumulation of sediments by the ^{210}Pb dating technique is a feasible process. In Bob Lake, if we assume that the two anomalous results are due to the coring operation, depth in the lake alone explained 97% of the between-site variation in sediment accumulation rate. If this single relationship can be confirmed in other lakes, future estimates of total annual accumulation of sediments can be derived from a relatively small series of cores provided all samples are good and the upper limit of sediment accumulation is carefully ascertained.

For studies designed to test our simple hypothesis, a sampler such as the K–B corer will be inadequate, first because there is no guarantee that it collects a vertical core, and second because the shallow areas with coarse bottom materials in which it functions poorly constitute a large fraction of the lake basin. Although sediment accumulation is certainly slow in this area, the absence of samples from it introduces uncertainty into the final estimate which in Bob Lake was equal to ±9%. Probably, if we are to be certain that sampling is adequate, it will have to be done by divers or with devices thoroughly tested by diver-observers. We stress this because sampling, although the least time-consuming part of this work, is the part most subject to uncontrolled error.

Despite the fact that we have stressed the weakness of our data, the comparison of our estimate of P accumulation rate in Bob Lake with the estimate obtained by the mass budget method is most encouraging. The first estimate of P retention in Bob Lake was made by Dillon (1974), who measured the major inputs and output over 18 mo. He calculated that average retention is 115 mg·m^{-2}·yr^{-1}. Subsequently Cross (1977) repeated the study in greater detail and concluded that P retention is 140 mg·m^{-2}·yr^{-1}. Dillon measured only the major inputs and assumed that the many, small, temporary streams entering Bob Lake had a low P export of 2.5 mg·m^{-2}·yr. Cross sampled these streams and showed that because their P concentration is high as they dry up, they export from 3 to 14.4 mg·m^{-2}·yr^{-1}. We therefore believe that 140 mg·m^{-2}·yr^{-1} is the better figure with which to compare our range of 143–170 mg·m^{-2}·yr^{-1}.

Considering the number of untested assumptions and potential sources of error in our estimate of P accumulation rate, the similarity between the two results is surprising. In fact the two results are well within the range of error that would be expected from the nutrient budget method. One cause of uncertainty in the results of the nutrient budget method is caused by our inability to measure aeolian loading accurately. It is often a significant fraction of total loading, and estimates of it vary by a factor of two depending on the type of fallout collector used (Gomolka 1975). Also, in a cottaged lake, such as Bob Lake, no method has been developed to measure the input of P from cottages. If cottages do contribute P to the lake, it is an input that was unmeasured by Cross (1977) and Dillon (1974). The omission of this input would cause both studies to underestimate true retention. Obviously more careful comparisons are needed before we can have confidence in the accuracy of either method, but we believe that our initial comparison shows that measurements of accumulation rate of sediments for whole lake areas and related element retentions are feasible using lead-210 dating.

Acknowledgments

We thank P. Cross for the use of sediment cores; H. Evans, who determined the wet mass, dry mass, and phosphorus concentrations; D. Skraba, who performed many of the ^{210}Pb analyses; and Dr G. Brunskill for helpful suggestions regarding the manuscript. This work was supported by an NSERC grant to FHR.

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Spring Transition Period in Lake Ontario — A Numerical Study of the Causes of the Large Biological and Chemical Gradients[1]

DONALD SCAVIA AND JOHN R. BENNETT

Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, Ann Arbor, MI 48104, USA

SCAVIA, D., AND J. R. BENNETT. 1980. Spring transition period in Lake Ontario — a numerical study of the causes of the large biological and chemical gradients. Can. J. Fish. Aquat. Sci. 37: 823–833.

A two-dimensional model that calculates physical transport, as well as in situ biological and chemical transformations, accurately simulates observations made along a north–south transect in Lake Ontario during April–June 1972. Simulation results show that, during the transition period between spring and summer, the inshore–offshore structure of biological and chemical distributions is controlled by the interaction of in situ processes and differences in vertical mixing on either side of the 4° isotherm. Owing to reversals in flow patterns, the effect of advection is to reduce concentration gradients, but the effect on overall distributions is minimal. An analysis of sinking losses in one- and two-dimensional models indicates that the artificially low sinking rates used in one-dimensional models of the Great Lakes result from the neglect of upwelling.

Key words: Lake Ontario; model, hydrodynamic, ecological; sinking, upwelling, convection cells, chemical distributions

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Un modèle à deux dimensions calculant le transport physique ainsi que les transformations biologiques et chimiques in situ simule avec précision les observations effectuées le long d'une coupe nord-sud du lac Ontario entre avril et juin 1972. Les résultats de cette simulation démontrent que, durant la période de transition printemps–été, la structure, du rivage vers le large, des distributions biologiques et chimiques est réglée par l'interaction de processus in situ et de différences de mélange vertical de part et d'autre de l'isotherme de 4°. Par suite d'inversions de la circulation, l'effet de l'advection est de réduire les gradients de concentration, mais l'effet d'ensemble sur les distributions est minimale. L'analyse des pertes par plongée des eaux dans des modèles à une et deux dimensions indique que les taux de plongée artificiellement bas utilisés dans les modèles à une dimension des Grands Lacs résultent du fait qu'on ne tient pas compte de la remontée d'eaux profondes.

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THE spatial distribution of biological and chemical properties in the Great Lakes is determined by variations of water depth, sunlight, and temperature; by the locations of rivers; and by currents. Separating these physical factors from in situ transformations is a difficult problem, a problem which numerical simulation can help solve.

Stadelmann and Fraser (1974) described seasonal variations in nitrogen and phosphorus and proposed a simple nutrient cycle model to describe biological/chemical processes in the summer — average epilimnion along a north–south transect of Lake Ontario. However, they did not examine detailed hydrodynamic effects. Using a three-dimensional simulation, Simons (1976a) examined the importance of physical processes in Lake Ontario. He found vertical mixing to be more important than currents in spring, but currents to be dominant in summer and fall. But, he did not compare the physical with the biological/chemical processes. We will do that here.

We choose to focus on the transition period between spring and summer because both vertical mixing and large-scale circulation are important. In addition, if we limit ourselves to this period, the temperature and current patterns of Lake Ontario are relatively two-dimensional (i.e. small longshore gradients). We can therefore make the assumption that variations along the long axis of the lake are negligible. Here we simulate the flow, temperature, biology, and chemistry for a north–south transect of the lake. Our approach is as follows.

The temperature calculations of a hydrodynamic model (Bennett 1971, 1974) were compared to ob-

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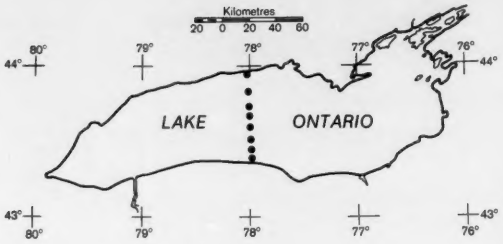

FIG. 1. Lake Ontario showing location of sample stations along the north–south transect under investigation.

servations, and the model was adjusted until computed and observed temperature contours were in general agreement. We then repeated the calculations, this time including the chemical and biological processes from an ecological model (Scavia et al. 1976; Scavia 1980), and compared them to observations. Only the sinking rate in the ecological calculations was adjusted to obtain good agreement between computed and observed variables.

In the remaining sections of this paper we will describe the transect data and model input for the hydrodynamic and ecological models, compare the observations with the model, and then analyze the effects of sedimentation and transport versus in situ processes.

Data and Models

Data used in this study were collected during the International Field Year for the Great Lakes (IFYGL) in 1972–73. Observations of temperature, nutrients, and biomass variables for our study were obtained from STORET, the data management system of the U.S. Environmental Protection Agency, and are based on collections made by the Canada Centre for Inland Waters (Stadelmann and Fraser 1974). The north–south transect used here follows approximately the 78°W longitude (Fig. 1) and has eight stations with between 5 and 10 depths sampled at each. A total of nine cruises was conducted, but we will analyze only the spring warming period: three cruises conducted during April 12–14, May 24–26, and June 20–22. Methods of data collection and measurement can be found in Stadelmann and Fraser (1974), Stadelmann and Munawar (1974), and Vollenweider et al. (1974).

Input data for the model include solar radiation and photoperiod (percent sunlit day) for phytoplankton calculations and heat flux and wind stress for hydrodynamic calculations. Incident solar radiation varied between 160 and 320 W/m^2 and was input as weekly averages from Atwater and Ball (1974). Photoperiod varied between 52 and 64% of the day.

The hydrodynamic model is driven by lake-averaged values of wind stress and net heat flux. The wind stress

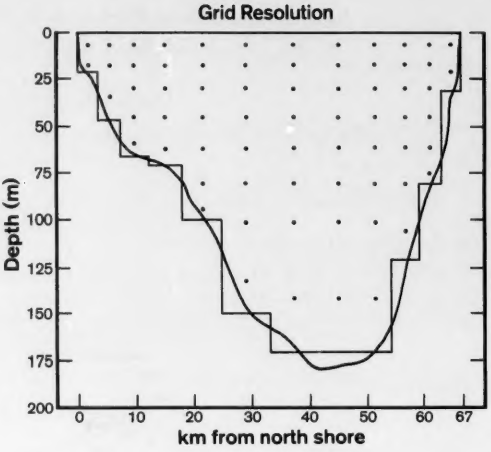

FIG. 2. Lake Ontario, north–south transect, two-dimensional model grid. Transect is approximately along 78°W longitude. Dots represent centers of model grid boxes.

was calculated from all available IFYGL meteorological buoys with a wind stress coefficient (the product of a drag coefficient and the air–water density ratio) of 2.5×10^{-6}. We used the heat flux estimates Boyce (1975) made from lake temperature measurements as did Simons (1976a, b). The coefficients of vertical viscosity and diffusion are a constant value, 32 $cm^2 \cdot s^{-1}$, modified by the Munk–Anderson functions of the Richardson number. But, when density decreases with depth the diffusion coefficient is increased to a value large enough that temperature and all ecological parameters are vertically mixed. Model calculations were performed with a time step of 2 h along a stretched grid (Fig. 2) that emphasizes resolution of the nearshore and surface. This grid is coarse, but we repeated the calculations using one half the grid size in both directions and found only small differences.

The ecological framework of this model was developed previously for simulation and analysis of a two-layer, lake-wide-averaged Lake Ontario system (Scavia et al. 1976; Scavia 1980). The crude physical segmentation in this previous work was intentional; it reduced the complexity of physical processes and allowed investigations with emphasis on biological, chemical, and simple physical processes at a lake-wide-averaged scale. Ecological calculations performed within the present physical context are the same as those calculated for the cruder segmentation. Detailed documentation of the system equations, demonstration of the ability of the model to simulate lake-wide average conditions, and examination of biological and chemical processes on that scale can be found in Scavia (1979, 1980). What follows are general descriptions

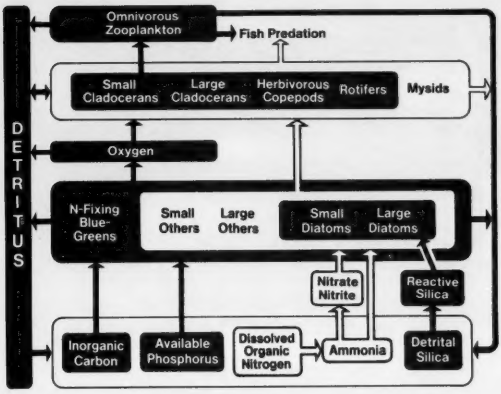

FIG. 3. Components of the ecological portion of the model (from Scavia 1980). Arrows indicate flow of carbon, phosphorus, nitrogen, and silica.

of the biological and chemical processes included in the model.

The ecological portion of the model (Fig. 3) predicts phytoplankton, zooplankton, cycles of phosphorus, nitrogen, silicon, and carbon, and oxygen balance; and calculations of carbonate equilibrium. Each model compartment is represented by a differential equation for the biological and chemical processes. For example, the phytoplankton equation includes terms for gross primary production, respiration, excretion, grazing, and sinking; and the zooplankton equation includes terms for grazing assimilation, respiration, excretion, defecation, and predation.

Gross phytoplankton production is considered a single-step process that assumes that, for the time scale of the modeled processes, rates of uptake and growth are in equilibrium. The process is modeled with a temperature-dependent maximum growth rate times a reduction factor for light- and nutrient-limitation. Potential light-limitation is modeled after Vollenweider (1965) and Steele (1965) and potential nutrient-limitation is expressed as a Michaelis–Menten term for each nutrient. The threshold formulation is used to determine overall limitation. Phytoplankton respiration is considered to be the sum of a low maintenance rate plus a term proportional to production and temperature. To maintain constant nutrient stoichiometry within the plankton, we set nutrient excretion proportional to respired carbon.

Zooplankton grazing is handled as a temperature-dependent saturation expression based on total food supply. The expression includes a minimum threshold for feeding as well as resource partitioning based on size selection. Feeding and assimilation efficiencies are regarded as food-specific constants. Food ingested but not assimilated is egested as detritus. Respiration is a temperature-dependent process that is the sum of a low maintenance rate and a term proportional to the feeding rate. Nutrient excretion is proportional to respiration.

Transformation rates between detritus, dissolved organic nitrogen, ammonia, nitrite plus nitrate, available phosphorus, and available silicon pools all are temperature-dependent and first-order. Sinking rates of phytoplankton and detritus are size- and density-dependent.

Results

Physical Properties

During the spring transition period a combination of strong heating and low wind speeds causes the thermocline to form. Because the lake begins the spring colder than 4°C, the temperature of maximum density, this process starts in the shallow water. Thus, the lake is divided into two hydrodynamic regimes — a deep region where the water temperature is less than 4°C and where surface heating causes vertical mixing, and a shallow region near the coast with temperature greater than 4°C, which may stratify.

In Fig. 4 the simulated temperature is compared with observations. The model correctly simulates this general spring temperature pattern and the depth of the thermocline. In addition, the fact that the thermocline extends further off the north shore than the south is also reproduced. There are two reasons for this: the north shore is shallower than the south and for the last 3 wk of the simulation the wind was from the west, causing upwelling on the north shore and downwelling on the south shore.

Wind and buoyancy combine to cause simple but interesting flow patterns. The wind tends to drive a one-cell pattern, with upwelling at the shore to the left of the wind and downwelling near the opposite shore. Heating drives a two-cell pattern, with the warm water rising near both shores and colder water sinking in the deep region.

Figure 5 shows the mean circulation for three, 24-d periods and the mean for the entire 72-d simulation. During the first two 24-d periods the wind came predominantly from the east; because the Coriolis force deflects the water to the right, the water near the surface flows north and the deep water flows south in compensation. Upwelling near the south shore and downwelling near the north shore closes this circulation. During the last 24-d period the wind and circulation reverse. For the whole 72-d period the wind's effects tend to cancel and the circulation looks like the thermally driven pattern.

This simple picture of a combination of wind and buoyancy effects should be considered an average circulation pattern. At any given time the flow is dominated by the wind, and it is only because the thermally driven flow is more persistent that it is as important as the wind-driven flow. The average longshore flow, generated

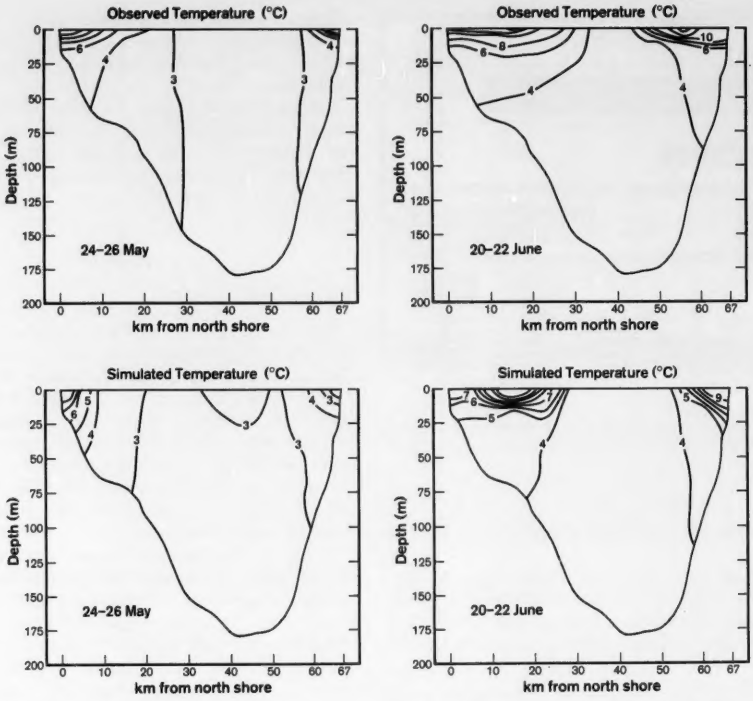

FIG. 4. Comparison of observed (*upper*) and calculated (*lower*) temperatures (°C) corresponding to May and June cruises.

by the Coriolis force, is cyclonic, to the east along the south shore and to the west along the north shore.

ECOLOGICAL PROPERTIES

Using the ecological equations and parameters unchanged from the original lake-wide-average, two-layer version resulted in comparisons between observations and computations that were generally good for plant nutrients (phosphorus, nitrogen, silica) but poorer for estimates of biomass (chlorophyll *a*, particulate organic carbon). By the end of the simulation period, model estimates of biomass in the nearshore surface areas were 1.5 to 2.5 times higher than observations (about June 20). We increased the average phytoplankton sinking rate by one order of magnitude over that used in the simple two-layer model, from ~0.05 to 0.5 $m \cdot d^{-1}$. This resulted in better agreement between observations and calculations. This simple modification improved the simulation of both nutrients and biomass variables.

In general, nutrient concentrations had slight offshore gradients and were vertically homogeneous in early April. These data were used to initiate the simulation. By the end of May, distinct offshore gradients had developed: nutrients, especially phosphorus and silica, were severely depleted in the regions within 10 km of both shores (Fig. 6). Also, at this time, no strong vertical gradients were obvious in either the model output or the data. At the end of the period of simulation (corresponding to the June 20–22 cruise) the symmetry of the north- and south-shore contours was lost. The region of nutrient depletion along the north increased to greater than 25 km and vertical stratification was not as strong there. The spatial and temporal progression of the region of nutrient depletion is demonstrated for phosphorus in Fig. 6; inorganic nitrogen and soluble reactive silica have similar patterns. The comparison between observed total dissolved phosphorus (TDP) and modeled available phosphorus (AP) is made because previous simulations of phosphorus cycling in Lake Ontario (Scavia 1980) indicated that in spring AP is approximated best by TDP, due, presumably, to production of easily hydrolyzed phosphorus compounds during the previous winter.

Biomass parameters (chlorophyll *a* and particulate organic carbon) in April were relatively homogeneous

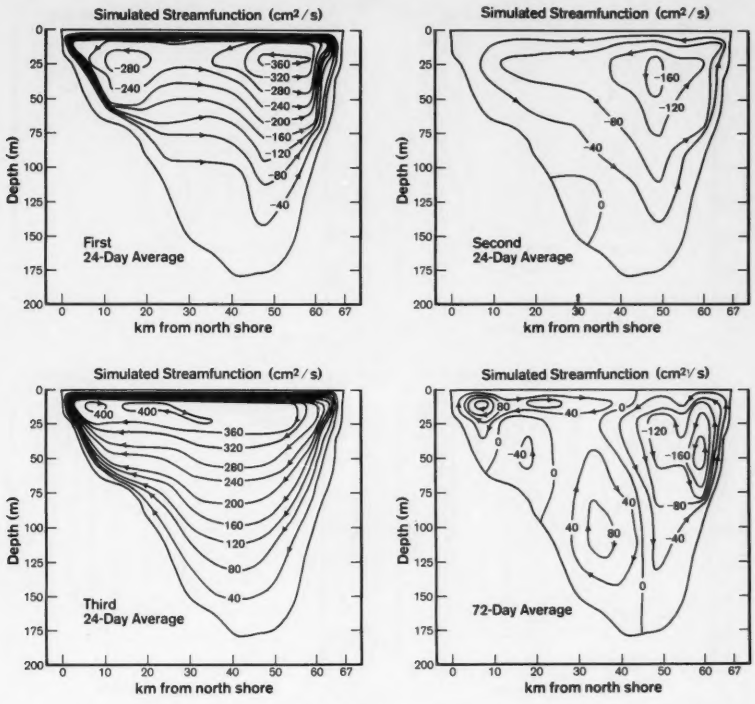

FIG. 5. Contours of calculated stream functions (cm^2/s) averaged over three 24-d periods and over the entire period of simulation.

vertically, with higher values nearshore. Patterns in May generally showed offshore gradients and little vertical structure (Fig. 7), except for evidence of nearshore subsurface chlorophyll peaks, which the model did not reproduce. The simulations indicated that between the May and June cruises, upwelling moved the higher nearshore concentrations offshore, creating a lens of high biomass about 15 km from the north shore. (See Fig. 4, 5). By the time of the June cruise, increased nearshore production apparently created higher biomass again close to shore. A similar structure was produced along the south shore with the exception that, like the nutrient contours, the biomass contours were constrained closer to shore by the wind-driven flow.

Discussion

In the analysis of the progression and simulation of nutrient and biomass isopleths, two important considerations became clear: (1) the impact of sinking on biomass and the formulation of that process in various models: (2) the relative influence of physical transport versus in situ processes. These are discussed below.

SINKING

Parameterization of plankton sedimentation in models of the Great Lakes' ecosystems has proved difficult. Sinking rates used in these models have had to be considerably lower than rates measured in laboratory studies. Sinking rates of algae, within typical size ranges of Lake Ontario plankton (Stoermer and Ladewski 1978), are summarized in Table 1. From available information one would expect the free-fall settling rates for Lake Ontario phytoplankton to be within 0.4–1.2 $m \cdot d^{-1}$. It has been recognized (e.g. Thomann et al. 1975; Scavia 1980) that sinking rates used in one- or two-layer, horizontally averaged Great Lakes' models (Table 2) are considerably lower than rates measured under quiescent laboratory conditions; however, if these measured sinking rates were used in the calculations, loss rates from the upper mixed layer would be too great to allow sufficient net biomass production in the models. Rates 10 or 20 times lower are required to obtain better agreement between observations and calculations of both phytoplankton standing crops and total nutrient loss to the sediment (Scavia 1980; DiToro and Matystik 1979).

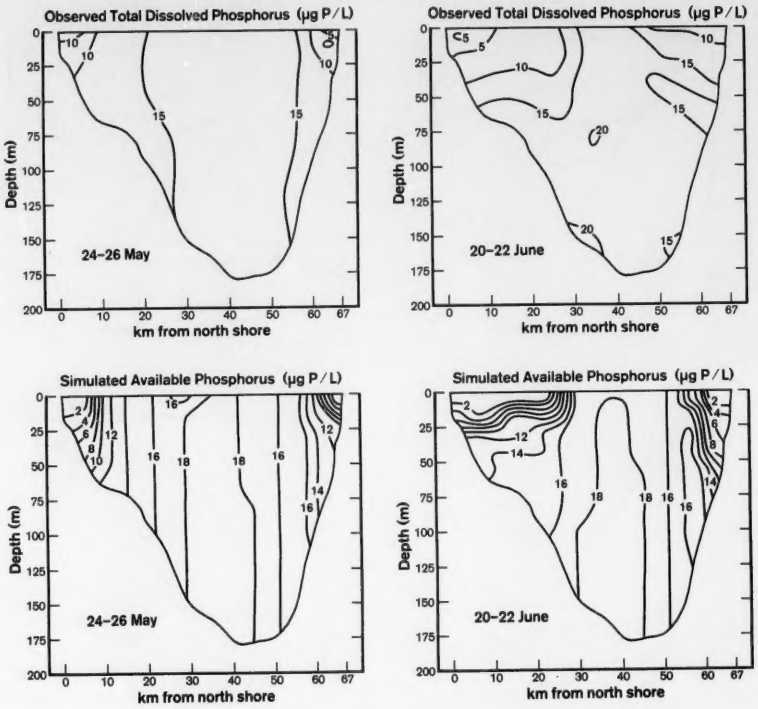

FIG. 6. Comparison of observed (*upper*) and calculated (*lower*) concentrations of phosphorus (μg P/L) corresponding to May and June cruises. Observed phosphorus is total dissolved phosphorus and calculated phosphorus is that considered available for phytoplankton growth.

As discussed above, the transect model required use of more realistic sinking rates ($0.5\ m \cdot d^{-1}$). Using the typical "one-dimensional" sinking rates ($0.05\ m \cdot d^{-1}$) resulted in poorer agreement between observations and calculations. In fact, there was little difference between using a rate of $0.05\ m \cdot d^{-1}$ and eliminating sinking entirely.

To explore the reasons for the discrepancies between measured sinking rates, rates used in one-dimensional models, and rates used in the present work, we examined some of the important phenomena that can affect sinking: physiological state, shape, size, colony formation, and water movements. Sinking rates appear to vary with physiological state only by factors of 2–4 (Titman and Kilham 1976; Smayda 1970, 1974; Eppley et al. 1967). Typical shape corrections for Stokes settling of nonspherical particles are at most a factor of 2 (McNown and Malaika 1950; Hutchinson 1967). Size and colony formation also are not sufficient to account for the reduced rates.

For water movements to affect particle sedimentation significantly, there must be a correlation between areas of upwelling and high biomass concentrations. The correlation would result in a net upward transport of particulates and would consequently require lower sinking rates in one-dimensional models, which cannot account explicitly for such vertical water movements. Although the wind-driven circulation in our two-dimensional model produces upwelling and downwelling on opposite shores (Fig. 5a–c), the composite average circulation over the entire period of simulation (Fig. 5d) has rising motion on both shores. These areas of net upwelling are in fact associated with areas of higher biomass concentrations (Fig. 7) and thus one would expect a net upward transport opposing sedimentation.

The association of higher biomass concentrations and these nearshore zones may be partially explained by plankton responses to higher nearshore temperatures, to higher rates of nutrient supply in the nearshore upwelling zones, or to some other fortuitous set of phenomena that may not persist beyond this particular spring transition period.

Another cause for higher plankton concentrations

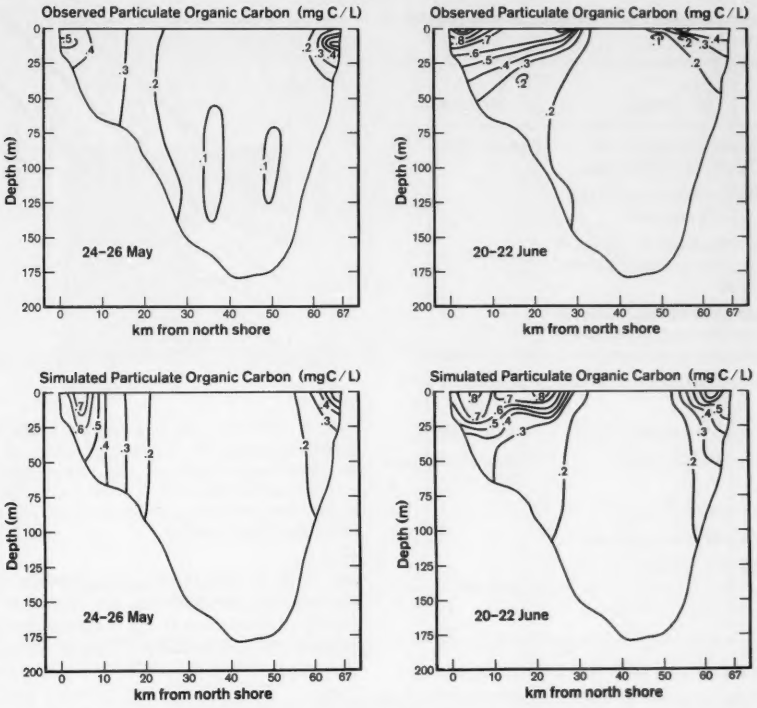
Observed Particulate Organic Carbon (mg C/L)
Observed Particulate Organic Carbon (mg C/L)
Simulated Particulate Organic Carbon (mg C/L)
Simulated Particulate Organic Carbon (mg C/L)
Depth (m)
24–26 May
20–22 June
km from north shore

FIG. 7. Comparison of observed (*upper*) and calculated (*lower*) concentrations of particulate organic carbon (mg C/L) corresponding to May and June cruises. Calculated carbon is the sum of simulated phytoplankton, zooplankton, and detrital carbon.

in these nearshore zones may be entrapment of particles within zones of retention of large-scale convection cells set up during upwelling. This phenomenon has been discussed often with regard to Langmuir circulation (e.g. Stommel 1949; Smayda 1970; Titman and Kilham 1976); however, the grid of our model cannot resolve this smaller-scale circulation. If the larger-scale vertical circulation patterns predominant in our study are analogous to Langmuir cells, then their effects on sinking can be estimated.

Titman and Kilham (1976) demonstrated the difference in sinking loss rates calculated by using a simple algebraic relationship (Table 3, Eq. 1) and by using an estimate of loss from a Langmuir cell (Table 3, Eq. 2), where a critical parameter is the fraction of a population that is outside the zone of retention set up by the Langmuir circulation. Smayda (1970) cites Koppen (1921), who suggests that the fraction of mixed-layer plankton entrained in the vortex of a vertical convection cell can be approximated by equation (3) (Table 3). This relationship was derived originally by Schmidt (1913) for particles sinking through a turbulent atmosphere and was applied to the development of hailstones. The relationship can be derived by calculating the ratio of areas of two discs of rotation defined by the stream functions of a convection cell and a sinking particle trapped within it. Substitution of equation (3) into equation (2) results in the definition of loss from a vertical convection cell (Eq. 4).

By dividing equation (1) by equation (4) and using typical values for the parameters, we can examine the relationship between sinking losses within a convection cell and in the absence of such water movement (Eq. 5, Table 3). With a mixed layer depth of 10–20 m, upwelling speeds of 10 $m \cdot d^{-1}$ (typical maximum speeds from the transect model), and a loss rate from the zone of retention of 0.02 d^{-1}, we obtain results similar to those of Titman and Kilham (1976) for Langmuir cells. That is, if the same sinking rate were used in equations (1) and (4), the algebraic loss rate (L_a) would be 10–30 times greater than that calculated as loss from a convection cell (L_c). Also, under the above conditions, to affect similar loss rates in both contexts, the sinking rate used in equation (1) (i.e. one-dimensional models) must be $\frac{1}{10} - \frac{1}{30}$ that used in equation (2).

The final test of the hypothesis that unrealistic sink-

TABLE 1. Ranges of sinking rates of algae of sizes typical of Lake Ontario plankton.

Rate ($m \cdot d^{-1}$)	Comment	Reference
0.2–0.5	Marine unicellular, size based on cell diameter	Smayda 1970
0.2–1.4	Marine chains, size based on cell diameter	
0.1–2.0	All cells (marine), size based on surface area to volume ratio	
0.1–1.0	Marine discs, size based on surface area to volume ratio	
0.17–1.7	Marine, sized based on cell volume	Vinogradova 1977
0.26–1.04	*Scenedesmus quadricauda*	Titman 1975
1.26	*Tabellaria fenestrata*	Titman and Kilham 1976
0.1–1.5	*Asterionella formosa*	
0.08–0.24	*Cyclotella meneghiniana*	
0.67–1.87	*Melosira agassizii*	

TABLE 2. Plankton sinking rates used in Great Lakes' models.

Lake/Bay	Rate ($m \cdot d^{-1}$)	Reference
Grand Traverse Bay	0.02–0.05	Canale et al. 1976
Saginaw Bay	0.05	Bierman et al. 1979
Lake Ontario	0.03–0.05	Scavia 1980
Lake Ontario	0.1	Thomann et al. 1975
Lake Erie	0.05	DiToro and Connolly 1979
Saginaw Bay–Lake Huron	0.05	DiToro and Matystik 1979

TABLE 3. Equations[a] comparing sinking losses calculated as simple algebraic loss and as loss from a convection cell.

(1) $L_s = v/Z$

(2) $L_c = D \cdot f$

(3) $1 - f = (1 - v/V)^2$

(4) $L_c = D\frac{v}{V}\left(2 - \frac{v}{V}\right)$

(5) $\frac{L_c}{L_s} = \frac{ZD}{V}\left(2 - \frac{v}{V}\right)$

[a] L_s = algebraic sinking loss, L_c = sinking loss from convection cell, v = sinking rate, Z = mixed-layer depth, V = upwelling speed, D = loss rate from convection cell, f = fraction of area (volume) outside zone of retention for a particle sinking in a convection cell.

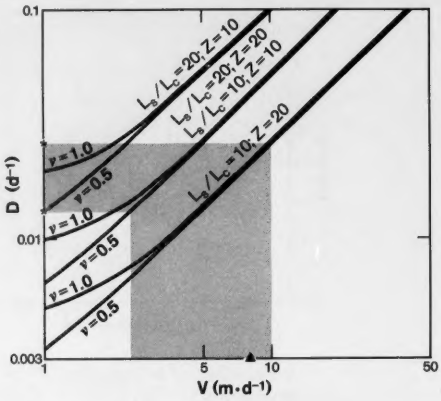

FIG. 8. Plot of turbulent mixing coefficient (D) versus upwelling speed (V) for various values of the ratio L_s/L_c, the mixed layer depth (Z), and sinking speed (v). Asterisks (*) define the range of D from Lake Ontario dye experiments. The solid triangle represents typical upwelling speeds calculated in the model.

ing rates in one-dimensional models are a result of the need to parameterize the effects of water circulation is to determine if the required horizontal loss rate of 0.02 d^{-1} is reasonable for the scales under consideration. Kullenberg et al. (1973) performed dye experiments in Lake Ontario to estimate coefficients of turbulent mixing. They reported horizontal diffusivities for both the region of the epilimnion and the region of the thermocline and the hypolimnion. These diffusivities, divided by the square of the mixing lengths (3–10 km) under consideration, are a rough estimate of the horizontal loss rate. These calculations result in values of D (d^{-1}) between 0.013 and 0.026; thus, a value of 0.02 d^{-1} is reasonable. The relationship between L_c and L_s in terms of D and V, from equation (5), is shown in Fig. 8. Here we examine specifically the case of one-dimensional versus two-dimensional models of Lake Ontario; thus, we know sinking rates were increased from 0.05 to 0.5 $m \cdot d^{-1}$, respectively. If the rates were not adjusted, we would expect an L_s:L_c ratio of about 12:1 (Eq. 5, Table 3). In Fig. 8 we plot D versus V for $L_s/L_c = 10$ and 20, and Z and v relevant to Lake Ontario. It can be seen that to obtain correct L_s/L_c ratios with appropriate values of v and Z and with D from Kullenberg et al. (1973), upwelling speeds (V) would have had to have been 2–10 $m \cdot d^{-1}$. This is the range of values obtained during our two-dimensional simulations.

From this analysis we suggest that reduced sinking rates are required in one-dimensional models of the Great Lakes partially because entrainment of phytoplankton in large-scale circulation, in a fashion similar to entrainment in Langmuir cells, effectively reduces their overall loss rate.

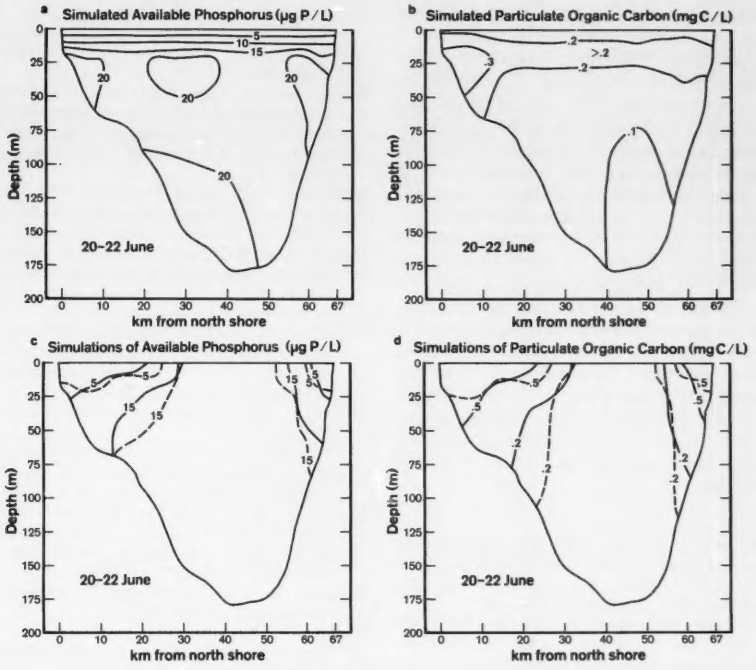
a Simulated Available Phosphorus (μg P/L)
b Simulated Particulate Organic Carbon (mg C/L)
c Simulations of Available Phosphorus (μg P/L)
d Simulations of Particulate Organic Carbon (mg C/L)
Depth (m)
km from north shore
20–22 June

FIG. 9. Contours of model output demonstrating the relative effects of physical and biological processes on the distributions of available phosphorus (μg P/L) and particulate organic carbon (mg C/L). a, b — contours of model output generated with no advection or diffusion included; c, d — broken lines represent normal case (i.e. all processes included); solid lines represent simulation without vertical or horizontal advection.

HYDRODYNAMIC TRANSPORT VERSUS IN SITU PRODUCTION

During spring there are several physical mechanisms that influence the distribution of chemical and biological parameters. Differences in surface temperature can stimulate different rates of biomass production and nutrient cycling. Differences in vertical temperature gradients can produce different intensities of vertical mixing. Currents driven by density differences or wind stress can bring nutrients into the photic zone.

Munawar and Munawar (1975) suggest that vertical mixing is the most important physical mechanism in determining the distribution of diatoms in the nearshore region of Lake Ontario. Menon et al. (1971) found more bacteria on the shore side of the 4° isotherm, but did not suggest a reason. Gachter et al. (1974) explained that differences in offshore versus inshore nutrient concentrations in Lake Ontario were due to deep vertical mixing in the offshore water.

We performed some numerical experiments to find out which of the physical mechanisms was most important. In these experiments we ran two simulations and compared the results to the original calculations discussed above (henceforth, the normal case). In the first simulation, we eliminated mass transport by advection and diffusion. In the second simulation, only vertical diffusion was included. In all cases in situ biological and chemical processes and sinking were included and temperature distributions were kept the same as in the normal case. The results of these experiments are summarized in Fig. 9 for available phosphorus and particulate organic carbon.

The simulation with no advection or diffusion showed that phytoplankton production in the cold offshore waters, although reduced when compared to inshore waters, is sufficient to deplete nutrients from the surface layer within the 72-d simulation (Fig. 9a) Therefore, temperature-controlled, in situ production alone was not sufficient to produce the persistent offshore gradients. Also evident in this experiment (Fig. 9b) were plankton concentration peaks in the second model layer (10–20 m) rather than in the surface layer, as in the normal simulations and in the observations. Depth pro-

files such as these are typical of lakes that experience little turbulence (e.g. Hutchinson 1967, p. 286).

The second simulation illustrates that vertical mixing, together with in situ production, is sufficient to reproduce the observed biological and chemical patterns. The major effect of vertical and horizontal advection is to smooth the nutrient gradients through increased mixing caused by repeated reversals in flow direction. The same was true for plankton along the relatively dilute, cold-water boundary (Fig. 9d).

It appears that the distribution of chemical and biological properties in the vicinity of the 4° isotherm is controlled primarily by the interaction of in situ processes and the differences in vertical mixing on either side of the isotherm. Shoreward, the water mass is weakly stratified vertically. This reduces the mixed depth and allows increased biomass production and subsequent nutrient depletion (Sverdrup 1953; Stadelmann et al. 1974). Lakeward, deep vertical mixing keeps a significant portion of the phytoplankton removed from the sunlit surface layers and therefore inhibits their growth.

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Relationships Between Metabolic Parameters and Stream Order in Oregon

ROBERT J. NAIMAN[1] AND JAMES R. SEDELL[2]

Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR 97331, USA

NAIMAN, R. J., AND J. R. SEDELL. 1980. Relationships between metabolic parameters and stream order in Oregon. Can. J. Fish. Aquat. Sci. 37: 834–847.

The light regime, standing stock of chlorophyll, and five metabolic parameters were measured seasonally at four Oregon stream sites; Devils Club Creek (1st order), Mack Creek (3rd order), Lookout Creek (5th order), and the McKenzie River (7th order). Periphyton from pools and riffles, aquatic moss (*Fontinalis*), fine particulate organic matter (FPOM: 0.5 μm–1 cm), and coarse particulate organic matter (CPOM: >1 cm) were examined separately for gross production (GP), net community production (NCP), diel respiration (R_{24}), net daily metabolism (NDM), and the production to respiration (P:R) ratio. Total autotrophic production was found to increase in a downstream direction where more light is available; however, the efficiency of light utilization is greatest in heavily shaded Mack Creek. Detrital metabolism, per unit weight, is similar in all streams for each metabolic parameter. The standing stock of detritus though, is highest in headwater streams, decreasing as streams become larger. Therefore, the relative contribution of the detritus community to total metabolism decreases downstream. Mosses occur in significant quantities only in the McKenzie River and have an areal community respiration rate about twice that of periphyton; other metabolic parameters are nearly equal in this case. On an areal basis periphyton metabolism is much greater than detrital metabolism and, as a consequence, total stream metabolism and the P:R ratio increase downstream.

Gross production rates measured for these sites are some of the lowest recorded (0.1–1.0 g $O_2 \cdot m^{-2} \cdot d^{-1}$). These rates are compared to those from other streams in the northwestern United States and western Canada, and the importance of photosynthesis to stream systems is demonstrated. The decomposition rate of the detrital standing stock is estimated to be 0.0029–0.0057% $\cdot m^{-2} \cdot d^{-1}$ and the turnover time is calculated to be 48–93 yr for particles <10 cm in diameter.

Key words: streams, rivers, metabolism, primary production, detritus, photosynthesis, chlorophyll, respiration, light, Oregon

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Le régime lumineux, la biomasse de chlorophylle et cinq paramètres métaboliques ont été déterminés saisonnièrement à des sites de quatre cours d'eau de l'Orégon : ruisseau Devils Club (1er ordre), ruisseau Mack (3e ordre), ruisseau Lookout (5e ordre) et rivière McKenzie (7e ordre). Nous avons examiné séparément le périphyton des fosses et des radiers, la mousse aquatique (*Fontinalis*), le matériel organique particulaire fin (FPOM : 0,5 μm–1 cm) et le matériel organique particulaire grossier (CPOM : >1 cm) afin d'en déterminer la production brute (GP), la production communautaire nette (NCP), la respiration nycthémérale (R_{24}), le métabolisme quotidien net (NDM) et le rapport production : respiration (P : R). On constate que la production autotrophe totale augmente vers l'aval où il y a plus de lumière; cependant, l'efficacité d'utilisation de la lumière est maximale dans le ruisseau Mack, fortement ombragé. Le métabolisme détritique, par unité de poids, est semblable dans tous les cours d'eau pour chaque paramètre métabolique. Cependant, la biomasse détritique est maximale dans les cours d'eau de tête et diminue à mesure que les cours d'eau grossissent. La contribution relative de la communauté détritique au métabolisme total diminue donc vers l'aval. Les mousses ne se trouvent en quantités importantes que dans la rivière McKenzie et ont un taux respiratoire communautaire par superficie environ deux fois plus élevé que celui du périphyton; les autres paramètres métaboliques sont dans ce cas à peu près égaux. Sur base de superficie, le métabolisme du périphyton est de beaucoup supérieur au métabolisme détritique et, comme conséquence, le métabolisme total du cours d'eau et le rapport P:R augmentent vers l'aval.

Les taux de production brute mesurés à ces sites sont les plus bas qu'on ait enregistrés

[1]Present address: Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.

[2]Present address: U.S. Forest Service, Forest Sciences Laboratory, 3200 Jefferson Way, Corvallis, OR 97331, USA.

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(0,1 à 1,0 g $O_2 \cdot m^{-2} \cdot jour^{-1}$). Nous comparons ces taux à ceux d'autres cours d'eau du nord-ouest des États-Unis et de l'ouest du Canada et démontrons l'importance de la photosynthèse dans les réseaux fluviaux. On estime à 0,0029–0,0057% $\cdot m^{-2} \cdot jour^{-1}$ le taux de décomposition de la biomasse détritique et calcule que la période de renouvellement est de 48 à 93 ans pour des particules de diamètres <10 cm.

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RELATIVE proportions of total metabolism by autotrophic and heterotrophic components of streams is largely a function of the physical dimensions of the stream channel, which affects the light regime, nature of allochthonous inputs, and discharge patterns. Normally, small headwater streams in forested areas are heavily shaded, and have considerable detritus associated with the substrate (Naiman and Sedell 1979, 1980). As stream size increases the forest canopy opens, allowing more light to reach the water surface, and correspondingly higher discharge rates transport detritus out of the stream channel and downstream. Extensive periphyton and macrophyte communities develop in these medium-sized streams, direct detrital inputs from the forest are less, and the metabolic importance of detritus to total stream metabolism is usually reduced over that in the headwaters. Further downstream, as the forest canopy opens completely and the river becomes deep and turbid, the autotrophic component is largely restricted to a phytoplankton community, while the mineralization of fine detritus being transported now plays a larger metabolic role (Cummins 1975, 1977; Vannote et al. 1980).

This generalization has been developed from studies of specific stream sites but has never been adequately tested in a single river system. Previous studies of community metabolism have been restricted to single reaches, usually only primary production or total metabolism have been measured, and because of the ease of sampling, these studies have been largely conducted on small streams. There is only one study of periphyton over several stream orders (Seyfer and Wihlm 1977), and few comprehensive studies of larger streams and rivers (Wetzel 1975; Schmidt 1976; Fisher 1977). Only Odum (1957), Teal (1957), Tilly (1968), Fisher and Likens (1973), Naiman (1976), and Fisher (1977) have attempted to isolate the various components of total metabolism in stream ecosystems, usually concentrating on springs and low-order streams.

In this study we examined gross production (GP), net community production (NCP), diel respiration (R_{24}), net daily metabolism (NDM), and the production to respiration ratio (P:R) of autotrophic and detrital components of stream segments ranging from a small headwater stream to a moderately large river. These data are used to test the hypothesis that the P:R ratio is <1 in small streams where there is considerable detritus and reduced light levels, the ratio increases to >1 in midorder reaches where the detrital standing crop is reduced and the forest canopy is partially open; and the ratio decreases to <1 as water depth increases, the river becomes turbid, and metabolism associated with drifting fine detritus increases in relative importance. Our measurements and observations were made on the McKenzie River system in western Oregon, USA.

Study Area

The McKenzie River originates in Oregon's Cascade Mountains. It is characterized by high gradient stream channels, a maritime climate, and considerable seasonal rainfall. All streams in the Pacific Northwest experience autumn and spring freshets and low summer flows; annual precipitation ranges from 225 cm at lower elevations to about 350 cm on ridges. High elevations have extensive snow pack during winter; rain predominates at lower elevations (Berntsen and Rothacher 1959). Dominant forest vegetation is Douglas fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*). Red alder (*Alnus rubra*) is associated with lower elevation streams. Three of the four study sites (Devils Club Creek, Mack Creek, and Lookout Creek) are within the H. J. Andrews Experimental Forest about 70 km east of Eugene, Oregon, and one (McKenzie River) is adjacent to the forest (Fig. 1). The dynamics of drifting organic matter (Sedell et al. 1978; Naiman and Sedell 1979) and benthic detritus (Naiman and Sedell 1980) have been previously reported for these streams.

Devils Club Creek is a first-order stream (Strahler 1957) heavily shaded by forest, and with a poorly developed autotrophic community (Table 1). The stream channel is choked with coarse organic debris derived from windfall Douglas fir (~400-yr old) accumulated over the last several hundred years, and leaf fall from huckleberry (*Vaccinium parvifolium*), devilsclub (*Oplopanas horridum*), maple (*Acer macrophyllum* and *Acer circinatum*), and Douglas fir needles (Naiman and Sedell 1979, 1980). Devils Club Creek discharges directly into Mack Creek.

Mack Creek is one of three major channels draining the Andrews Forest. The morphology of this third-order stream is characterized by a stairstep of gouged pools, free fall zones, and turbulent water around large boulders (Table 1). Substrate ranges from large boulders to sand but is mostly loose cobble (Sedell et al. 1975). Woody debris is prevalent but less concentrated than in Devils Club Creek (Anderson et al. 1978; Naiman and Sedell 1980). The forest canopy is slightly open and patches of light reach the stream at midday. Wind-borne debris, lateral movement from steep side

FIG. 1. A, Devils Club Creek; B, Mack Creek; C, Lookout Creek; D, McKenzie River. All study sites are located in the Cascade Mountains about 70 km east of Eugene, Oregon.

slopes, and downstream transport of organic matter from tributaries are the primary organic inputs. Mack Creek is a major tributary of Lookout Creek.

Lookout Creek (5th order), receiving runoff from most of the Andrews Forest, has shallow riffles interspersed with small pools; the substrate is large cobbles and bedrock. There is relatively little woody debris in the stream, the canopy is open, and most sunlight reaches the periphyton community. Primary production by diatoms (*Melosira*), filamentous algae, and stream lettuce (*Prasiola*), as well as transport from upland areas, are the major organic inputs (Naiman and Sedell 1979, 1980). Allochthonous inputs from maple leaves and conifer needles are important primarily only in late summer and autumn. Lookout Creek discharges into Blue River, a tributary of the McKenzie River.

The McKenzie River is a major seventh-order stream draining the western Cascade Mountains. Morphologically, it is 85% riffle-runs with a few alcoves or pools. Substrate is cemented cobble and large boulders; large amounts of woody debris have not accumulated (Table 1; Naiman and Sedell 1980). Alder and Douglas fir along stream margins contribute some organic matter but most inputs are from upstream transport, periphyton production, and aquatic macrophytes. A zone of aquatic mosses (*Fontinalis* sp.) extends 5 m from both banks.

Organic inputs vary widely in amount and composition for each study site (Naiman and Sedell 1979, 1980). All Andrews Forest sites have similar temperature regimes with winter lows of about 1°C and summer maxima of about 15 to 18°C, and similar water chemistry (Table 1; Naiman and Sedell 1979). The McKenzie River has a narrower annual temperature range varying from about 3°C in winter to 12°C in summer, and a slightly different water chemistry with

TABLE 1. Physical characterization of the Oregon study locations.

Parameter	Devils Club Creek	Mack Creek	Lookout Creek	McKenzie River
Stream order	1	3	5	7
Watershed area (km^2)	0.2	6.0	60.5	1024.0
Mean width (m)	0.6	3.0	12.0	40.0
Mean depth (m)	0.05	0.22	0.90	1.60
Benthic organic matter (g AFDW·m^{-2})	28 800	14 882	5 872	833
Forest canopy development	Closed	Mostly closed	Mostly open	Open
Mean annual discharge (L·s^{-1})	1.7	92	377	75 300
Benthic substrate	Detritus	Boulders–cobbles	Cobble	Cobble
Temperature range (°C)	1–15	1–16	1–18	3–12
Total chlorophyll (mg Chl a·m^{-2})	19–48	36–95	18–96	31–90
Nutrient range ($\mu g \cdot L^{-1}$)				
NO_3-N	0.3–23.5	3.4–76.0	0–43.2	0–4.2
PO_4-P	3.9–12.8	5.8–12.4	7.3–15.4	28.2–49.6
Alkalinity range (mmol·L^{-1})	0.063–0.137	0.056–0.132	0.013–0.131	0.167–0.244

higher concentrations of phosphorus, lower concentrations of nitrogen, and slightly higher alkalinity. All sites are quite different in stream flow, channel geometry, and basin morphometry (Table 1), but all have the exceedingly clear water typical of high montane salmonid streams.

Methods and Materials

General Approach

The major metabolic components of each stream were measured separately because a natural section of substrate could not be accommodated by the metabolic chambers. The autotrophic component was either mosses or periphyton on rocks; periphyton was further identified depending as to whether the rocks were from a riffle or pool. The heterotrophic component consists of animals and microbes associated with detritus. Animal metabolism is usually a small percentage of the total community metabolism (Naiman 1976) and was not measured in this study. Detritus was separated according to size; woody debris (CPOM) consisted of cones, bark, leaves, needles, and wood >1 cm in diameter. For this study only, fine particulate organic matter (FPOM) was considered to be smaller debris, generally <1 cm in diameter. Total stream metabolism was estimated for each site by combining all metabolic components on an areal basis.

In these streams large woody debris >10 cm in diameter (bole wood) is the major detrital component, comprising over 90% of the detrital standing crop. Nearly all of this material is in the form of trees up to 1 m in diameter (Naiman and Sedell 1980). Metabolism associated with this material was not measured because (1) much of it is in the stream channel but not under water, (2) it could not be realistically sampled and placed in the chambers, (3) and, although it is an important structural component of streams, it has a low biological activity, decomposing over very long time periods (Anderson et al. 1978).

Techniques and Procedures

Sampling was in March (winter), May (spring), July (summer) and October (autumn), 1976. Winter data for Devils Club Creek and Mack Creek are minimal because of heavy snow accumulations and severe weather that restricted access.

Periphyton, moss, and detritus were collected, placed in separate sealed recirculating Plexiglas metabolic chambers (volume: 14 L), and oxygen changes monitored with YSI oxygen meters connected to a strip chart recorder (Bott et al. 1978). All chambers were placed in the stream from which the samples had been collected, except for Devils Club Creek. In this instance the chambers were placed about 30 m away in Mack Creek because Devils Club Creek was too small to hold them. It is felt that no serious errors resulted from this since Devils Club Creek and Mack Creek have a similar water chemistry and temperature regime (Naiman and Sedell 1979), and a similar light regime. Metabolic parameters were measured by the diel oxygen method (Bott et al. 1978) using the following equations:

1) Gross primary production (GP) = net community primary production plus community respiration during photoperiod.
2) Net community primary production (NCP) = GP − R_{pp}.
3) Community respiration (R) = total respiration during photoperiod (pp) or diel period (24).
4) Net daily metabolism (NDM) = GP − R_{24}. The net change caused by community metabolism over a diel period.
5) P:R ratio = GP ÷ R_{24}.

Four chambers were run simultaneously for various periods ranging up to 96 h at each location. During winter and spring samples in the chambers were changed after 4 d, with each diel period considered to be a separate data point. In summer and autumn samples were replaced after 2 d with each day used as a separate metabolic measurement. Normally, all chambers were flushed every 4–8 h to avoid oxygen, nutrient, and CO_2 depletion stress. Metabolism of microbes on the Plexiglas was <5% ($n = 4$) of samples placed in the chambers, even after 4 d of continuous submergence. Incident radiation at the water surface was measured continuously with a LiCor model 185 quantum radiometer.

After each experiment the chamber contents were placed in containers, frozen, and returned to the laboratory. The

TABLE 2. Daily light regime ($\mu E \times 10^5 \cdot m^{-2} \cdot d^{-1}$) at the water surface for each sampling location during the metabolic experiments.

	Devils Club Creek		Mack Creek		Lookout Creek		McKenzie River	
Season	$\bar{x} \pm$ SE	n	$\bar{x} \pm$ SE	n	$\bar{x} \pm$ SE	n	$\bar{x} \pm$ SE	n
Winter	4.4	1	5.1	1	75.0±9.2	4	70.4±17.7	4
Spring	24.7±8.7	4	33.3±6.9	5	177.2±39.7	4	101.5±20.7	4
Summer	10.1±0.5	4	11.1±1.5	4	165.0±25.7	6	67.3±10.4	6
Autumn	2.7±1.0	3	2.7±0.8	4	98.1±9.5	4	130.0±28.9	4

ash-free dry weight (AFDW) of material in each chamber was determined by drying to a constant weight at 60°C and ashing a subsample at 550°C for 4 h. Prior to drying, chlorophyll was extracted by immersion in 1 L of 100% acetone for 24 h. Water associated with the samples reduced the final acetone concentration to about 90%. Chlorophyll *a* was calculated using the equation of Lorenzen (1967). Surface area of rocks was measured by wrapping each rock with aluminum foil, weighing the foil, and converting to area using the weight of foil per unit area. Of the total rock area, about 50% is photosynthetically available and 25% is available when viewed as a horizontal plane from above the stream (T. D. Bott, personal communication; Gregory 1979). Therefore, multiplying total rock area by 0.25 gave the photosynthetic area in m^2 of stream bottom.

It was not possible to measure the metabolism of the intact benthic community because of the wide range of particle sizes encountered, turbulence of the water, and spatial segregation of the metabolic components. Because of this results for each metabolically active component were combined to estimate total metabolism in the following manner. In chambers with only detritus, metabolism was measured in g $O_2 \cdot$ g $AFDW^{-1}$ of detritus per day. This value was combined with measurements of the seasonal standing stock of detritus <10 cm in diameter (Naiman and Sedell 1980) to estimate total detrital metabolism in g $O_2 \cdot m^{-2} \cdot d^{-1}$. This was added to metabolic measurements of periphyton on rocks to estimate total community metabolism. In the McKenzie River an estimate of total metabolism is complicated because of the moss (*Fontinalis*) component. Moss occurs over ~5% of the total area; therefore total metabolism was prorated as: 0.05 (moss metabolism) + 0.95 (periphyton metabolism) + detritus metabolism.

Concomitant with the oxygen measurements an effort was made to measure photosynthetic and respiratory quotients by the method of Talling (1973). Each time chambers were flushed, water samples were collected in stoppered containers and the alkalinity determined by Gran titration with 0.1 N HCl and a Coleman 80 pH meter. The results obtained were not considered reliable. Photosynthetic quotients ranged from −14.5 to 17.5, respiratory quotients ranged from −7.0 to 2.6, with nearly all values far from the predicted quotients of 1.25 and 0.85 for protein metabolism, respectively. Our attempts to measure the PQ and RQ will not be presented here because of these facts, although the problems we encountered are worthy of notation.

Results and Discussion

LIGHT

Light regime — There is a significant difference in the light regime among streams (ANOVA, $P < 0.05$). Little sunlight reaches the stream surface in Devils Club Creek and Mack Creek (Table 2). Highest light levels for both streams occur during spring before understory shrubs and small trees (huckleberry, devilsclub, and maple) develop their seasonal foliage. Lookout Creek and the McKenzie River are wide enough to have a relatively open canopy. The difference in the light regime between these latter two streams is caused mainly by the directional orientation of the sites and by spatial placement of the chambers and light meter. The channel of Lookout Creek is oriented east–west; the McKenzie River channel is oriented north–south. Also, the McKenzie River, because of its powerful current, necessitated that chambers be placed near to shore and, as a consequence, some shading by riparian vegetation did occur during summer and autumn that was not characteristic of most of the stream channel. In addition, for all streams, the daily light regime is quite variable because of weather common in this geographical region.

THE AUTOTROPHIC COMPONENT

Periphyton metabolism — There is a steady increase in gross production, net community production, and diel respiration by periphyton as stream size increases from 1st-order Devils Club Creek to 5th-order Lookout Creek. Lookout Creek and the McKenzie River have nearly identical metabolic rates (Fig. 2). Seasonal estimates of gross production range from 0.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in Devils Club Creek to about 0.9 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in the McKenzie River. Net community production ranges from zero to about 0.6 g $O_2 \cdot m^{-2} \cdot d^{-1}$, while diel respiration varies from −0.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in the Devils Club Creek to almost −0.7 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in the McKenzie River. Metabolism is generally lowest in winter and autumn for all sites except the McKenzie River where the lowest metabolic rate occurs during spring snowmelt.

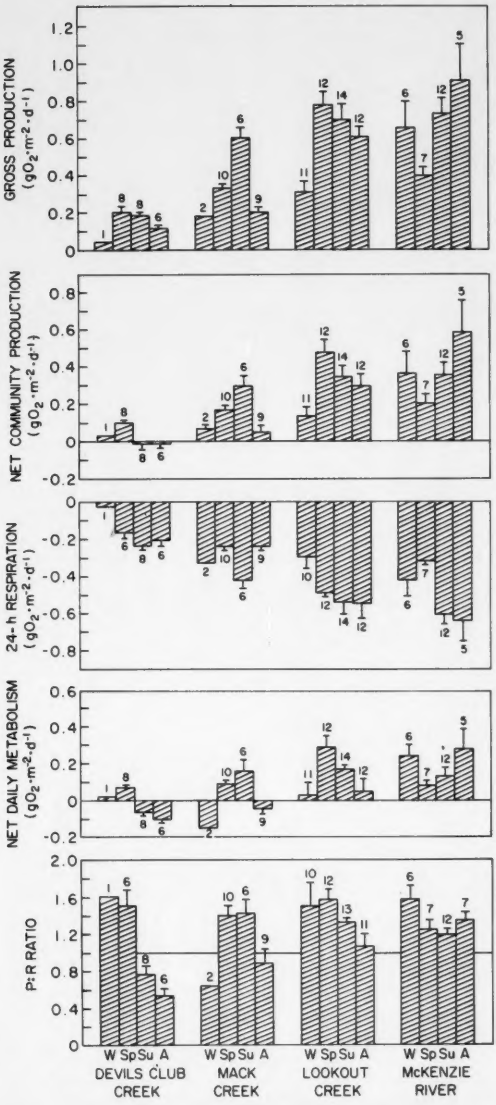

FIG. 2. Mean, SE, and sample size of measurements of gross production, net community production, diel respiration, and the P:R ratio of the periphyton community in each stream. Symbols are winter (W), spring (Sp), summer (Su), and autumn (A).

Net daily metabolism is lowest in Devils Club Creek (0.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$) and often negative (Fig. 2); at other sites it is generally positive, ranging up to 0.3 g $O_2 \cdot m^{-2} \cdot d^{-1}$, and of nearly equal magnitude for each

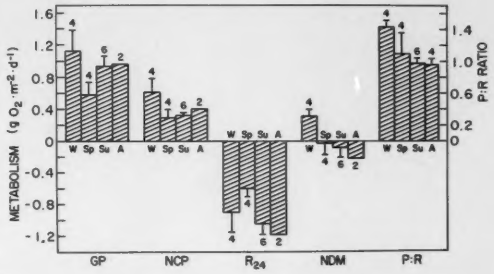

FIG. 3. Metabolism by moss in the McKenzie River compared by season. Mean, SE, and sample size are shown. Symbols are the same as in the previous figure.

stream. In this case there are no definite seasonal trends; highest values are in winter and spring for the McKenzie River; spring and summer in Lookout Creek; winter, spring, and summer in Mack Creek; and spring for Devils Club Creek.

P:R ratios have only a slight trend by stream order; each stream has periphyton P:R ratios >1 for at least part, if not all of the year (Fig. 2). The P:R ratio in Devils Club Creek is >1 in spring; the winter period should be viewed with caution since only one sample was obtained. Mack Creek exhibits a P:R ratio during spring and summer that is equal to or greater than those measured for periphyton in Lookout Creek and the McKenzie River.

Moss metabolism — Gross production, net community production, and diel respiration remain nearly constant throughout the year for the *Fontinalis* dominated community in the McKenzie River, except for a slight decrease in the respiration rate and, consequently, gross production during the spring freshet (Fig. 3). Gross production ranges from 0.6 to 1.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$; net community production ranges up to 0.6 g $O_2 \cdot m^{-2} \cdot d^{-1}$; and diel respiration ranges from about −0.6 to −1.2 g $O_2 \cdot m^{-2} \cdot d^{-1}$. Net daily metabolism is positive during the winter period and slightly negative at other times. However, standard errors for spring and summer estimates indicate accrual of organic matter is possible during these periods. The P:R ratio approximates unity throughout the year.

Chlorophyll — The standing stock of chlorophyll *a* in each stream is the same order of magnitude, ranging from 18 to 96 mg Chl $\cdot m^{-2}$, although Devils Club Creek is lowest for all seasons (Table 3). Surprisingly, heavily shaded Mack Creek has more chlorophyll at times than Lookout Creek or the McKenzie River. Our results are comparable to those reported by Lyford and Gregory (1975), who found concentrations ranging from 8 to 35 mg Chl $\cdot m^{-2}$ in Mack Creek and 8 to 45 mg Chl $\cdot m^{-2}$ in Lookout Creek over a 1.5-yr period. The amount of chlorophyll at these sites is comparable to that measured in other streams of the northwestern

TABLE 3. Mean seasonal concentration ($mg \cdot m^{-2}$) of chlorophyll *a*, and ratio of optical density at 665 μm before and after acidification, of rocks and detritus in each stream.

		Devils Club Creek			Mack Creek			Lookout Creek			McKenzie River					
											Periphyton			Mosses		
Season		*n*	$\bar{x} \pm$ SE	%	665/665a	*n*	$\bar{x} \pm$ SE	%	665/665a	*n*	$\bar{x} \pm$ SE	%	665/665a	*n*	$\bar{x} \pm$ SE	665/665a
Winter	Rocks	1	12.3	66.7	1.41	2	36.0	99.4	1.73	7	18.3±2.2	100.0	1.59	4	58.1±11.5	93.4	1.63	2	202.8	1.66
	Detritus	2	6.2	33.3	1.00	2	0.2	0.6	1.65	2	0.0	0	1.32	3	4.1±4.0	6.6	1.45			
	Total		18.6				36.2				18.3				62.2					
Spring	Rocks	2	18.8	88.7	1.58	3	49.5±5.2	91.2	1.66	3	59.7±5.6	97.5	1.75	2	31.1	99.7	1.61	1	28.9	1.12
	Detritus	2	2.4	11.3	1.43	1	4.8	8.8	1.53	1	1.5	2.5	2.29	1	0.1	0.3	1.51			
	Total		21.2				54.3				61.2				31.2					
Summer	Rocks	3	22.7±3.6	78.8	1.61	4	84.3±11.9	88.5	1.67	6	39.7±7.5	98.8	1.64	4	53.8±9.3	99.4	1.61	2	276.2	1.64
	Detritus	4	6.1±3.9	21.2	1.54	4	11.0±3.8	11.5	1.57	2	0.5	1.2	1.74	2	0.3	0.6	1.92			
	Total		28.8				95.3				40.2				54.1					
Autumn	Rocks	4	26.1±3.1	54.8	1.74	4[a]	61.9±13.6	95.8	1.67	6	96.0±11.9	99.7	1.66	4	89.1±11.9	99.6	1.62	2	441.2	1.65
	Detritus	4	21.5±17.1	45.2	1.36	3	2.7±2.0	4.2	1.61	2	0.3	0.3	1.55	2	0.4	0.4	1.63			
	Total		47.6				64.6				96.3				90.3					

[a]Excluding a measurement of 312.2 mg Chl·m^{-2} (665/665a = 3.15) from a chamber containing rocks covered with mosses.

United States and western Canada (Stockner and Shortreed 1976; Lane 1965; Cushing 1967).

The ratio of gross production to the standing stock of chlorophyll *a*, used here as an assimilation number, is markedly different between the lower- and higher-order streams (Table 4). In Devils Club Creek and Mack Creek, the seasonal ratio was generally between 3 and 8 while in Lookout Creek and the McKenzie River it ranged from 10 to 18. The ratios for the larger streams are similar to those found by McConnell and Sigler (1959) for a mountain river in Utah; the ratios for Devils Club Creek and Mack Creek are much lower. However, most of the assimilation numbers reported here are within the general range of 4.8 to 48.0 given by Odum et al. (1958) for whole communities.

Mean seasonal chlorophyll concentrations are significantly correlated with four of the five metabolic parameters (Table 5); only the P:R ratio does not have a significant correlation ($r = 0.08$). Best relationships are between gross production, net community production, and diel respiration. Why the P:R ratio does not have a significant correlation with chlorophyll is intriguing. Chlorophyll concentrations are roughly similar for all streams (Table 3) suggesting differences in metabolic rate are largely determined by light availability. Therefore, given a similar temperature regime, it appears standing stock of chlorophyll may determine the basic level of metabolism, while light strongly influences the active level of metabolism, as well as relative proportions involved in growth and respiration.

Efficiencies and correlations — It is evident that each stream, regardless of its size or light regime, has an active autotrophic community. Although the importance of the autotrophic community is relatively slight in Devils Club Creek, the P:R ratio often exceeds unity. Periphyton has more sites available for attachment in Mack Creek, and it is more important here, with some metabolic parameters nearly equal to those in Lookout Creek and the McKenzie River. These observations raise two questions: (1) Is the active level of periphyton metabolism a function of available light? (2) Are periphyton communities in shaded situations more efficient in their utilization of light? These questions can be partially answered by examining comparative efficiency of light utilization.

There is a significant difference in gross light efficiency between streams (ANOVA, $P < 0.05$) but not among season ($P > 0.05$) (Table 6). Gross efficiency of light utilization [(Gross production/g cal light) 100] in Devils Club Creek is normally about 1% but may be as high as 4% in autumn. In Mack Creek efficiency ranges seasonally from <1 to 5%, while efficiencies in Lookout Creek are about 0.5% and in the McKenzie River about 1%, suggesting that periphyton from shaded streams are more light efficient than periphyton from streams where the canopy is open. However, factors not studied here, such as herbivore grazing and scouring during freshets may be just as

TABLE 4. Mean gross production (GP) and net daily metabolism (NDM) per milligram of chlorophyll *a* in the periphyton component, for each stream by season. Units are mg $O_2 \cdot$mg $Chl^{-1} \cdot d^{-1}$.

Season	Devils Club Creek		Mack Creek		Lookout Creek		McKenzie River	
	GP	NDM	GP	NDM	GP	NDM	GP	NDM
Winter	3.6	1.5	5.1	4.2	17.0	2.0	11.4	4.2
Spring	10.5	3.8	6.7	1.8	13.1	4.8	12.8	2.6
Summer	7.8	−2.8[a]	7.1	1.9	17.7	4.3	13.8	2.4
Autumn	4.1	−3.9[a]	3.2	−0.7[a]	6.4	0.5	10.3	3.1

[a]Negative value caused by a net loss of organic matter over the diel period.

TABLE 5. Correlations between mean seasonal chlorophyll *a* concentrations and mean seasonal metabolic parameters for the periphyton community in all streams. Metabolic parameter units are mg $O_2 \cdot m^{-2} \cdot d^{-1}$; chlorophyll is $mg \cdot m^{-2}$. $n = 16$; $^*P < 0.05$; $^{**}P < 0.01$.

Metabolic parameter	Correlation (r)	Equation
Gross production (GP)	0.77**	$\text{Log}_{10}(\text{GP}) = 0.81 + 1.07 \log_{10}(\text{chl})$
Net community production (NCP)	0.69**	NCP = 4.78 (chl) − 8.11
Diel respiration (R_{24})	−0.73**	$R_{24} = -132.34 - 4.81$ (chl)
Net daily metabolism (NDM)	0.48*	NDM = 2.13 (chl) − 3.73
P:R ratio	0.08	

important as light availability in determining standing stock of chlorophyll and the level of periphyton metabolism in natural streams (Tett et al. 1978).

Attempts to correlate available sunlight with the five metabolic parameters at a single site gave correlation coefficients (r) generally <0.4 and significant at $P = 0.05$ in only 7 of the 20 possible cases (five parameters in four streams). The narrow range of sunlight values recorded for any one stream, in combination with seasonal variability due to temperature, chlorophyll standing crop, and discharge regime appeared to be primary causes for these results. When data for all streams are combined, the relationship between light and metabolism becomes clearer (Table 7). For each metabolic parameter the relationship is highly significant ($P < 0.001$) but the correlation coefficients are not large and only 17 to 41% of the variation in the measured metabolism can be attributed to variations in the light regime. This indicates that other factors, such as current, scouring, chlorophyll, and temperature, are also important in determining periphyton metabolic rates. The relative proportion of production to respiration (P:R:), however, is more a function of light than chlorophyll. Chlorophyll is not significantly correlated with P:R ($r = 0.08$, Table 5), while the correlation with light is highly significant ($r = 0.41$, $P < 0.001$, Table 7).

When mean seasonal gross production is scaled to a unit of chlorophyll (assimilation number) and regressed against mean seasonal sunlight (e.g. using light data collected during the seasonal experiments) there is a highly significant relationship ($r = 0.87$, $P < 0.001$) (Fig. 4). Nearly 76% of the stream to stream variation in the assimilation number can be attributed to light and nearly all the differences in gross production are caused by the interaction of light and chlorophyll.

TABLE 6. Calculated gross efficiency (%) of light utilization by periphyton.

Season	Devils Club Creek	Mack Creek	Lookout Creek	McKenzie River
	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE
Winter	0.9	2.3	0.3±0.05	0.8±0.16
Spring	0.8±0.26	0.9±0.21	0.7±0.11	0.3±0.08
Summer	1.3±0.07	3.6±0.44	0.3±0.02	0.8±0.12
Autumn	4.2±1.41	4.9±0.72	0.4±0.04	1.0±0.53

Autotrophic comparisons — Mack Creek can be characterized as having moderately delineated pools and riffles while in Lookout Creek they are well separated. The other sites are almost entirely riffles. Biological differences between pool and riffle habitats in terms of species, chlorophyll, and diversity are well known. Hynes (1972) and others (e.g. McIntire 1966) have demonstrated the effects of current speed on periphyton metabolic processes. In Mack Creek, depending upon the season, pool periphyton metabolism can be lesser or greater than riffle periphyton (Table 8). Pools here are small and, at high flows, essentially become riffles. On the other hand, in Lookout Creek pool periphyton metabolism is usually only 40–80% of that measured for riffle periphyton. However, for gross pro-

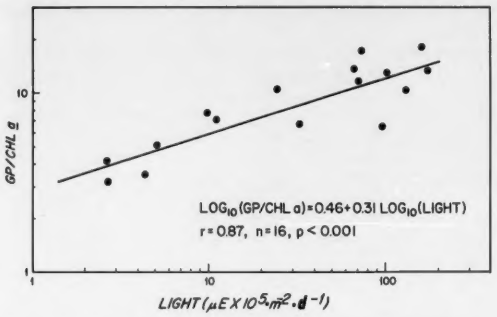

FIG. 4. The positive relationship between available sunlight and the assimilation number (GP/Chl *a*) using seasonal averages for each stream.

duction, there is a statistically significant difference between habitats only when spring and summer data are combined and compared to combined winter and autumn data *t*-test, $P < 0.05$). When all pool data are compared to all riffle data a significant difference is not present. It is interesting though, that in Lookout Creek the pool P:R ratio is equal to or greater than the riffle P:R ratio for three of the four seasons. In calculating the total periphyton metabolism of Lookout Creek the data were prorated by seasonal pool:riffle percentages; the other sites were not treated in this manner.

Mosses are a conspicuous component of the McKenzie River system but are thought to yield less material to higher trophic levels than periphyton primarily because of higher maintenance costs due to accrued biomass. *Fontinalis* is a perennial macrophyte with the biomass taking several years to accumulate. Compared to periphyton, gross production, net community production, and diel respiration by mosses are 17 to 83%

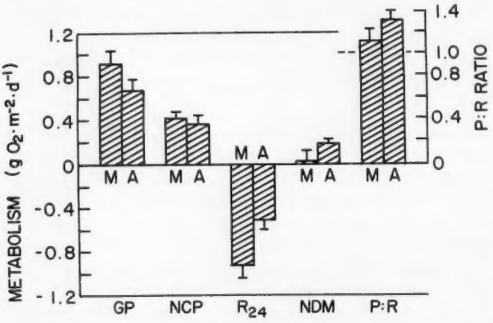

FIG. 5. Mean annual metabolism by moss (*Fontinalis*) and periphyton in the McKenzie River for each metabolic parameter. Symbols are moss (M), periphyton (A), gross production (GP), net community production (NCP), diel respiration (R_{24}), net daily metabolism (NDM), and the production to respiration ratio (P:R).

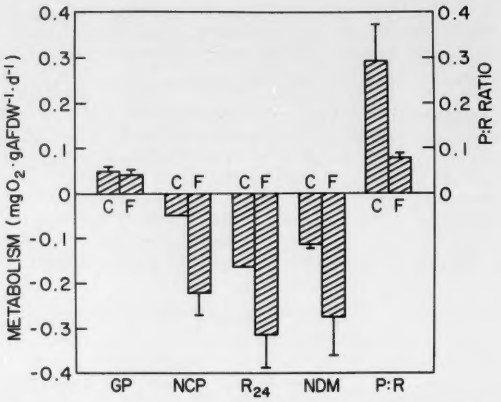

FIG. 6. Mean annual metabolism by coarse particulate organic matter (C) and fine particulate organic matter (F). Key to most symbols is given in Fig. 5; AFDW is ash-free dry weight.

greater (Fig. 5). However, most of the gross production of the moss community is due to its respiration. The result is that net daily metabolism by periphyton is about 550% greater than mosses and the P:R ratio is slightly higher for periphyton, suggesting that periphyton is the more significant of the two in directly determining overall river productivity.

THE DETRITUS COMPONENT

CPOM and FROM — Respiration rates are a function of particle size and, hence, surface area available for microbial colonization (Hargrave 1972; Naiman and Sedell 1980). These latter studies, restricted to particles less than about 1.6 cm in diameter, indicated respiration rates of benthic detritus are inversely proportional to particle size. However, the relationship between particle size and respiration rate of material being transported by the current is not clear (Naiman and Sedell 1979). To date, no one has examined metabolism by detritus particles greater than about 1.6 cm in diameter. On a weight basis CPOM comprises >90% of total detrital matter in each stream (Naiman and Sedell 1980); however, it would be expected from the studies of Hargrave (1972) and Naiman and Sedell that metabolic activity of CPOM would be considerably lower than FPOM.

In addition to microbes, there can be considerable microalgae associated with detritus (Table 4) (Anderson et al. 1978). This will vary from a few percent of the total stream chlorophyll (Lookout Creek and McKenzie River) to as much as 45% in Devils Club Creek. CPOM, because of its size, provides a relatively stable substrate for algal attachment and growth. Algae can also be attached to FPOM detritus particles but the stability of those particles is far less than for CPOM, and

TABLE 7. Equations relating metabolic parameters to surface sunlight for all streams and seaons combined. Sunlight units are microEinsteins ($\mu E \times 10^5$); metabolic units are mg $O_2 \cdot m^{-2} \cdot d^{-1}$.

Metabolic parameter	Sample size (n)	Equation	Correlation coefficient (r)	Significance
Gross production	129	$\text{Log}_{10}(\text{GP}) = 0.350 \log_{10}(\mu E) - 0.98$	0.64	$P < 0.001$
Net community production	127	$\text{Log}_{10}(\text{NCP}) = 0.073 \log_{10}(\mu E) - 0.03$	0.59	$P < 0.001$
Diel respiration	126	$\text{Log}_{10}(R_{24}) = 0.211 \log_{10}(\mu E) - 0.80$	0.50	$P < 0.001$
Net daily metabolism	126	$\text{NDM} = 0.130 \log_{10}(\mu E) - 0.11$	0.46	$P < 0.001$
P:R	127	$\text{Log}_{10}(\text{P:R}) = 0.135 \log_{10}(\mu E) - 0.16$	0.41	$P < 0.001$

TABLE 8. The ratio of metabolism by pool versus riffle periphyton for the five metabolic parameters measured in Mack Creek and Lookout Creek each season.

	Mack Creek							Lookout Creek						
Season	GP	NCP	R_{24}	NDM	P:R	Pool samples (n)	Riffle samples (n)	GP	NCP	R_{24}	NDM	P:R	Pool samples (n)	Riffle samples (n)
Winter	0.7	2.4	0.4	0.1	1.9	1	1	0.8	0.8	0.5	3.3	1.3	4	7
Spring	0.8	0.5	1.1	0.2	0.7	3	7	0.7	0.6	0.8	0.4	0.8	4	8
Summer	1.5	2.4	1.0	17.6	1.6	4	2	0.6	0.6	0.6	0.6	1.0	10	4
Autumn	1.9	72.0	0.9	−0.6[a]	2.2	3	6	1.0	1.0	0.8	−5.4[a]	1.3	6	6

[a]Negative value caused by a negative measurement for riffle periphyton.

the degree of shading from other particles is certainly greater. Depending upon the local situation photosynthesis by algae associated with detritus can be significant.

Gross production per unit of weight is nearly identical for both CPOM and FPOM (Fig. 6). However, net community production, diel respiration, and net daily metabolism of CPOM are only 23–53% of the FPOM metabolic rate. Conversely, the CPOM P:R ratio is nearly four times greater than FPOM. This is primarily due to more periphyton being associated with larger particles. These results indicate that CPOM and FPOM components of detrital metabolism must be treated separately in determining total stream metabolism.

Total detritus — Metabolism by the detrital community will either be a function of total standing crop at each site or influenced by stream to stream differences in metabolic activity per unit weight. There are no significant differences ($P > 0.05$) in metabolism per unit weight of detritus between streams and there are no seasonal trends; only detritus from Mack Creek in summer has a higher than normal metabolic rate but considerable variability is associated with those estimates (Fig. 7). This substantiates our earlier results using a Gilson respirometer with small particle sizes, that similar particle sizes have similar respiration rates, per unit of weight, between sites (Naiman and Sedell 1980). Differences of the total detrital community metabolism between sites are therefore due to differences in the standing stock of detritus.

Combining metabolism per unit weight of detritus (Fig. 7) with the mean seasonal standing crop of CPOM and FPOM (Naiman and Sedell 1980) gives an estimate of the metabolic activity of the total detritus standing crop (Fig. 8). Gross production of microbial communities on detrital substrates is the same for all streams each season. However, net community production, diel respiration, and net daily metabolism are highest in Devils Club Creek and Mack Creek where there are the largest standing stocks of detritus. All metabolic rates are negative in these streams, indicating a net degradation of organic matter. In some cases metabolic rates measured in Lookout Creek and the McKenzie River are slightly positive but, in all cases, are only a fraction of those measured in the two smaller streams. P:R ratios are generally <0.2 for detritus in Devils Club Creek and Mack Creek, rising to the 0.2–0.6 range in larger streams where more light is available for photosynthesis by associated microalgae.

If we assume all degradation of CPOM and FPOM is due to microbial activity and that all respiration is involved in the mineralization of particulate detritus, then respiration rates associated with CPOM and FPOM can be used to estimate the turnover time and decomposition rate of each detrital component. Using a factor of 2.75 to convert oxygen to carbon, given the fact that detritus is ~50% carbon, and using the standing crop estimates from Naiman and Sedell (1980), we calculate that for CPOM <10 cm in diameter in all streams the decomposition rate is $0.0029\% \cdot m^{-2} \cdot d^{-1}$ and the turnover time is ~93 yr. For FPOM the decompo-

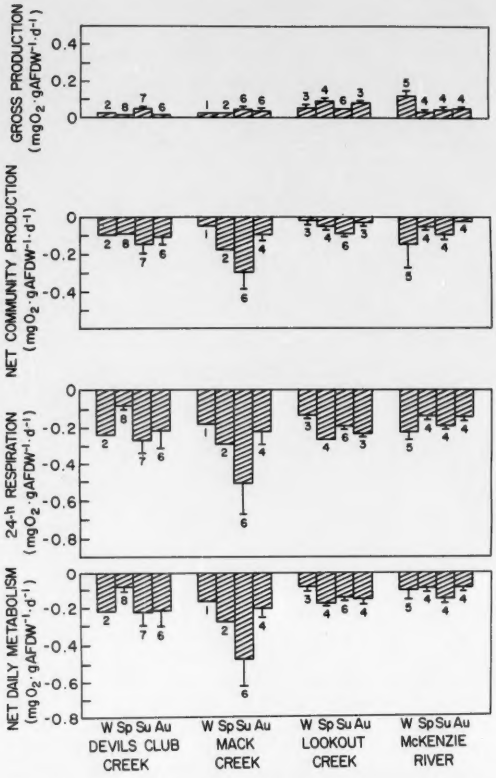

FIG. 7. Metabolism of detritus per unit of weight by site and season. Mean, SE, and sample size are given; symbols are described in previous figures.

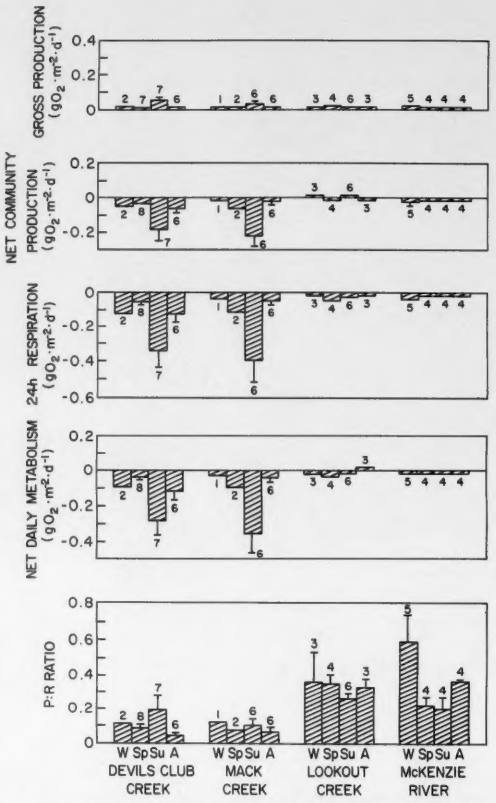

FIG. 8. Metabolism per unit of weight for detritus in Fig. 7 combined with the mean seasonal standing crop of CPOM and FPOM (Naiman and Sedell 1980) to estimate metabolic activity by the total detritus component. Symbols are described in previous figures.

sition rate is faster ($0.0057\% \cdot m^{-2} \cdot d^{-1}$) and the turnover time is shorter (~48 yr). If these estimates are realistic, it can then be expected that CPOM >10 cm in diameter would take considerably longer to be decomposed by microbial action, possibly 200–300 yr.

The time required for wood to decompose in freshwater has been estimated on only a few occasions. For woody debris in the streams studied here, Anderson et al. (1978) gave a degradation time of 5–200 yr with limited data on respiration rates. They did clearly show, however, that the role of invertebrates in wood decomposition is minor, giving a conservative estimate of $1–1.7\% \cdot yr^{-1}$. Hodkinson (1975) predicted a 57-yr half-life for 7.5-cm-diameter logs of *Populus balsamifera* in a cold water beaver pond in Alberta, Canada, and Fisher and Likens (1973) give a turnover time of 4.2 yr for small wood in Bear Brook, New Hampshire. The role of physical abrasion in the wood decomposition has never been evaluated, but it may be an important aspect of the process, especially since the role of microbes and invertebrates appears to be minor.

TOTAL STREAM METABOLISM

The highest metabolic rates for Devils Club Creek, Mack Creek, and Lookout Creek are in spring and summer when light levels and periphyton activity are near maximal (Fig. 9). Gross production ranges from about 0.2 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in Devils Club Creek to about 0.8 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in Lookout Creek and the McKenzie River. Overall, metabolic rates in Devils Club Creek and Mack Creek are only about half those measured in the larger streams, except for Mack Creek in summer when metabolism is equal to levels measured

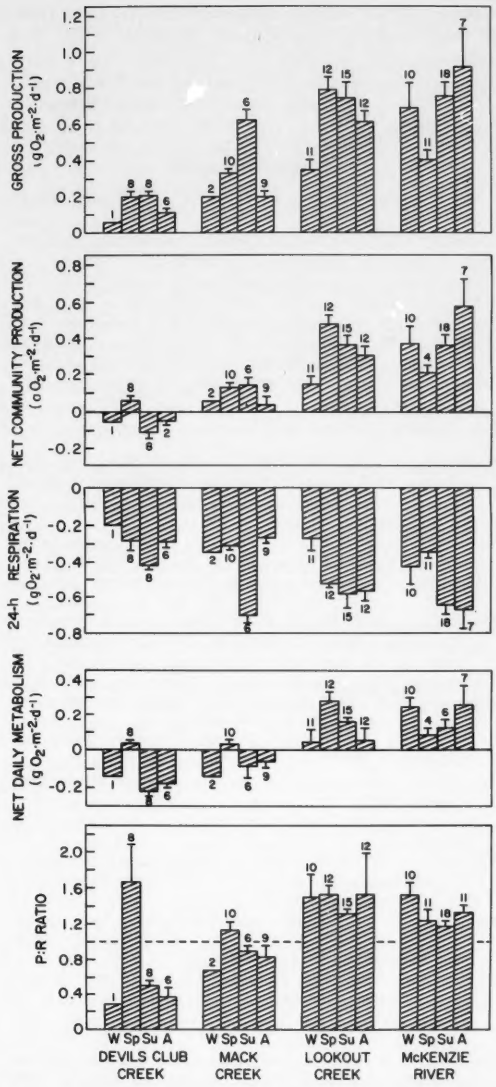

FIG. 9. Total metabolism by each stream on an areal basis estimated by combining the periphyton component with total detritus. Symbols are explained in other figures. Respiration by the animal community is not included; it is usually only a small part of the total.

in the largest streams. The McKenzie River has a relatively low metabolic rate in spring coinciding with peak discharge. Net community production and net daily metabolism are positive for all seasons in Lookout Creek and the McKenzie River (Fig. 9). Diel respiration generally increases in a downstream direction but differences between low- and high-order streams are not great; all sites respire between 0.2 and 0.7 g $O_2 \cdot m^{-2} \cdot d^{-1}$. Except during spring, when light levels are relatively high, Devils Club Creek and Mack Creek have P:R ratios <1. Lookout Creek and the McKenzie River have P:R ratios >1 with little annual variation.

SYNTHESIS

Comparisons with other streams — Streams examined in this study have some of the lowest production rates recorded to date but the values are comparable to other mountainous Oregon streams. Hansmann et al. (1971) reported gross production in Drift Creek, Oregon, ranging from 0.5 to 2.5 g $O_2 \cdot m^{-2} \cdot d^{-1}$, while Lane (1965) found gross production rates of 0.4 to 1.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in 2nd-order Needle Branch, Oregon, and 0.4 to 1.3 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in 3rd-order Flynn Creek, Oregon. Net production rates for Carnation Creek and Ritherdon Creek, British Columbia, two rain forest streams, are identical with rates in Devils Club Creek and Mack Creek (0.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$) and are possibly the lowest ever measured for a stream ecosystem (Stockner and Shortreed 1976).

Although not specifically studied here, some insights can be given to factors controlling metabolism in western Oregon streams. In all streams, metabolism will be affected by light and nutrient availability, temperature, and the hydrologic regime, with the relative importance of each factor varying from stream to stream. Major controlling factors in smaller streams are most likely the hydrologic regime and light availability. When forests associated with small streams are clearcut, light availability and nutrients are increased. Nutrient leaching is completed in a few years while light remains at increased levels for many years thereafter (Hansmann and Phinney 1973; Stockner and Shortreed 1976). Primary production in these stituations is enhanced remaining at relatively high levels several years after logging (Gregory 1979). Nutrients are dilute in the streams we studied (Naiman and Sedell 1979), probably limiting production in the larger streams where more light is available. McIntire and Phinney (1965) investigated effects of temperature on metabolism in streams finding dramatic changes with a ΔT of 10°C. Normally temperatures range from about 2 to 15°C during the year for all sites but total metabolism in these streams did not reflect these temperature changes, indicating the biota possibly undergo some temperature compensation with season.

Downstream trends in stream metabolism — From this study it is apparent that total benthic community metabolism per unit area increases as streams become larger, detritus metabolism per unit of weight remains the same regardless of stream size, total detrital metabolism decreases as streams become larger because

there is less detritus downstream, periphyton production increases with stream size because of greater light availability downstream, and the P:R ratio is >1 in downstream reaches, and can be >1 for short periods in the smaller streams depending upon the season. Total community metabolism is largely determined by the amount of detritus and associated periphyton in small streams, and mostly by the activity of periphyton alone in larger streams.

A good understanding of the community structure and physiological functions of stream ecosystems requires that major sources of organic matter and their fate within the system be known. At this time it is almost a paradigm that stream ecosystems are heterotrophic (Fisher and Likens 1973; Cummins 1974; Hynes 1975) despite extensive evidence that many streams can have an autotrophic base (Minshall 1978). Most communities in small streams appear to depend predominantly upon organic matter imported from adjacent terrestrial systems for their energy base because, to date, most ecological studies of streams have been conducted in temperate regions where forests provide abundant allochthonous energy sources and extensive shade, thereby appearing to minimize the importance of autochthonous photosynthesis. In small streams without extensive forest canopies or with suitable substrates for periphyton, or in larger streams where the forest canopy is open, autotrophic processes are important (Minshall 1978). Thus, all streams should not be considered heterotrophic, but rather the relative importance of heterotrophic and autotrophic processes should be critically evaluated. Minshall (1978) tabulated 40 P:R ratios finding values amost equally divided between those indicating heterotrophy and those indicating autotrophy. We summarized data from 27 streams finding that over a wide geographical area streams from orders 1 to 7 cannot be classified as either autotrophic or heterotrophic based on order alone. The relative importance of heterotrophy and autotrophy will depend upon the magnitude and character of detrital inputs, forest canopy development, the physical regime, and light availability.

Other trends seen in this study, such as total metabolism and P:R increasing in a downstream direction, have not been observed by other investigators but they have been postulated by Fisher (1977) and Vannote et al. (1980). Hoskin (1959) examined six sites in the Neuse River system, North Carolina, ranging from about a 2nd-order stream to approximately a 6th-order stream at the estuary, and only in the estuary was there a sharp increase in productivity. In this study metabolism and P:R showed no discernible trend with stream size. Likewise, in a short section of New Hope Creek, North Carolina, there was no seaward increase in gross production (Hall 1972).

Unfortunately, we did not choose a wide enough range of sampling stations to test completely the original hypothesis proposed by Cummins (1975, 1977). In general, the hypothesis appears to be correct for streams within a biome, ranging up to a moderately large river. It remains to be demonstrated, however, if the P:R ratio will again decrease to <1 in larger rivers where light attention due to depth and the metabolic activity of fine seston are major factors determining ecosystem metabolism.

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Dynamics of Selenium in Mercury-Contaminated Experimental Freshwater Ecosystems

JOHN W. M. RUDD, MICHAEL A. TURNER, BRUCE E. TOWNSEND, ALISON SWICK, AND AKIRA FURUTANI

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

RUDD, J. W. M., M. A. TURNER, B. E. TOWNSEND, A. SWICK, AND A. FURUTANI. 1980. Dynamics of selenium in mercury-contaminated experimental freshwater ecosystems. Can. J. Fish. Aquat. Sci. 37: 848–857.

The simultaneous movement of radiolabeled selenium and mercury was followed in experimental aquatic ecosystems ($\simeq 10^6$-L tube enclosures) set into a mercury-contaminated lake. The experimental treatments consisted of increasing stable selenium or sulfate water concentrations, addition of an aquatic herbicide, and isolation of a portion of the water column from contact with sediments. Selenium and mercury did not move together through the ecosystems either geochemically or biologically. Selenium bioaccumulation was rapid by fish, crayfish, and haptobenthos and was enhanced by increased sulfate concentration and in the absence of exposure to bottom sediments. It was reduced in the presence of aquatic herbicides. A selenium concentration of 0.1 mg/L did not affect the rate of movement of mercury out of the water column but it did stabilize the movement of mercury among various compartments in the water column. Elevated selenium appeared to retard the rate of mercury bioaccumulation by fish, crayfish, and haptobenthos. Preliminary observations of selenium toxicity to several aquatic organisms were carried out. The possibility of using selenium as an ameliorating agent for heavy metal polluted freshwater systems is discussed.

Key words: selenium, mercury, methylmercury, fish, crayfish

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Le mouvement simultané de sélénium et de mercure radiomarqués a été suivi dans des écosystèmes aquatiques expérimentaux (enceintes tubulaires d'environ 10^6 litres) placés dans un lac contaminé par le mercure. Les traitements expérimentaux ont été les suivants: augmentation des concentrations stables de sélénium ou de sulfate dans l'eau, addition d'un herbicide aquatique, isolement d'une portion de la colonne d'eau par rupture de contact avec les sédiments. Le sélénium et le mercure ne se déplacent pas ensemble dans les écosystèmes, ni géochimiquement ni biologiquement. La bioaccumulation du sélénium est rapide chez les poissons, les écrevisses et l'haptobenthos, et est favorisée par une augmentation de concentration de sulfates et par l'absence d'exposition aux sédiments du fond. Elle diminue en présence d'herbicides aquatiques. Une concentration de sélénium de 0,1 mg/L n'affecte pas le taux de mouvement du mercure hors de la colonne d'eau mais stabilise le mouvement du mercure parmi les divers compartiments de la colonne d'eau. Une concentration accrue de sélénium semble retarder le taux de bioaccumulation du mercure par les poissons, les écrevisses et l'haptobenthos. Nous avons fait des observations préliminaires sur la toxicité du sélénium chez plusieurs organismes aquatiques. Nous examinons la possibilité d'utiliser le sélénium pour améliorer les réseaux d'eau douce pollués par métaux lourds.

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MANY chemical and biochemical interactions of mercury and selenium have been observed in birds, fish, and mammals (Frost 1972; Frost and Lish 1975; Stadtman 1974; Wood et al. 1975; Sugiura et al. 1976; Chang et al. 1977; Kim et al. 1977; Sheline and Schmidt-Nielsen 1977). Selenium and mercury have been found to bioaccumulate in marine fish and mammals in at least a 1:1 at. ratio (Freeman et al. 1978; Smith and Armstrong 1978). Selenium has been found to have an antidotal effect on methylmercury intoxication by consumers of mercury-contaminated fish (Friedman et al. 1978; Ganther and Sunde 1974; Ganther et al. 1972; Ohi et al. 1976; Frost and Lish 1975). It has also been found to mobilize mercury from certain tissues resulting in redistribution of mercury among tissues and organs (Sumino et al. 1977; Sell 1977; Kim et al. 1977; Sheline and Schmidt-Nielsen 1977). Although the

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mechanism(s) of these interactions are poorly understood (Chang et al. 1977; Wood et al. 1975; Sumino et al. 1977), the existence of these interactions in vivo has been generally accepted.

With one exception (Hesslein et al. 1980) simultaneous movement of mercury and selenium in aquatic ecosystems has not been studied. Mercury–selenium interactions in aquatic ecosystems have never been investigated. Rates of selenium bioaccumulation and aquatic selenium toxicity are also almost unknown (Sandholm et al. 1973; Nielsen and Gissel-Nielsen 1978), as is the effect of elevated selenium concentrations on rates of mercury bioaccumulation.

During the summer of 1977 we carried out several experiments in $\simeq 10^5$-L enclosures (tubes) in Clay Lake, Ont., to observe movement of mercury and selenium and possible mercury–selenium interactions in fully functional aquatic ecosystems. Clay Lake has been severely contaminated by mercury during the past 17 yr (Armstrong and Hamilton 1973; Uthe 1972; Scott and Armstrong 1972; Scott 1974; J. W. M. Rudd et al. in preparation). The tube experiments described here were whole ecosystem experiments in which the ecosystems were altered in various ways in an attempt to manipulate and thereby elucidate the mechanisms of selenium and mercury bioaccumulation. In this way we hoped to be able to compare some aspects of the geochemistry of selenium and mercury, to examine the effect of elevated selenium concentrations on rates of mercury bioaccumulation by aquatic biota, and to follow the bioaccumulation and possible toxicity of selenium to the biota.

Methods

The experiments were conducted in six 10-m-diameter enclosures (tubes) in a sheltered bay of Clay Lake, northwestern Ont. (Armstrong and Hamilton 1973). The enclosure walls were constructed of cross-laminated polyethylene and were of a design similar to that described by Schindler et al. (1979). The walls of five tubes were sealed to the grey clay sediments at a depth of about 1.7–1.8 m. The remaining enclosure (bottom-sealed tube) had a watertight cross-laminated polyethylene bottom to eliminate sediment–water contact.

Enclosure Treatments

On day zero (July 15, 1978) each enclosure received 5 mCi $^{203}Hg(NO_3)_2$ (1 Ci = 37 GBq) and 2 mCi $Na_2{}^{75}SeO_3$ (New England Nuclear). Radioisotopes were used for these experiments for two reasons: because chemical methods would not be sensitive enough to discern treatment differences during the 8-wk duration of the experiments; and because isotopes enabled us to have a time zero with respect to monitoring effects of the enclosure treatments. A definite time zero was necessary to differentiate between selenium and mercury that was moving through the ecosystem before and after the enclosure treatments.

Selenium and mercury were added to the enclosures as selenite and mercuric ions because they are a common form of these elements in the aquatic environment. In addition, mercuric ion is an important substrate for mercury methylation, and one of the objectives of these experiments was to examine the ecosystem effect of added selenium on the rate of $CH_3{}^{203}Hg$ bioaccumulation in fish and crayfish muscle.

Table 1. Chronology of enclosure events.

Days after isotope addition	Event
−3 to −4	Tubes sealed to sediments and bottom-sealed tube filled
−3	Velpar® added to the herbicide tube (0.4 mg/L)
−2	First addition of Na_2SO_4 to the sulfate tube
−2	NaCl added to all tubes
0	Na_2SeO_3 and $Na_2{}^{75}SeO_3$ added to the selenium tube
0	$Na_2{}^{75}SeO_3$ and $^{203}Hg(NO_3)_2$ added to all tubes
3	Batch 1 pearl dace added to all tubes
16	Caged crayfish added to all tubes
24	Terbutryne® added to the herbicide tube (0.08 mg/L)
32	Second Na_2SO_4 addition to the sulfate tube
39	Batch 2 pearl dace added to all tubes
58	Termination of experiment

All the enclosures, including the controls, also received sufficient NaCl to double the ambient sodium and chloride concentrations. These ions were periodically monitored (Stainton et al. 1977) to assess possible enclosure leakage. No leakage was detected during the experiments.

Two of the tubes were experimental controls and received no other additions. Although two controls were used to give some indication of tube-to-tube variability, results from only one of the control tubes will be reported here since variability was not a problem in interpretation of results (J. W. M. Rudd et al. in preparation). Since regeneration of nutrients from sediments had been cut off in the bottom-sealed tube, weekly additions of $NaNO_3$ and NaH_2PO_4 were used to maintain the primary production activity at or above the level of the control tubes. Primary productivity was assayed by the ^{14}C incubator method of Fee (1973). One tube received two additions of sodium sulfate (Table 1) which increased sulfate concentrations from about 10 mg SO_4/L to about 46 and then 78 mg SO_4/L. Another received periodic additions of two aquatic herbicides, Velpar® and Terbutryne® (Table 1). The sulfate and herbicide treatments were primarily intended to alter bioaccumulation of mercury (Rudd et al. 1979). However, the treatments also affected selenium bioaccumulation. These data are reported here. To the sixth tube, sufficient stable sodium selenite was added to increase the selenite concentration in the water from undetectable levels (<0.2 μg Se/L) to 0.1 mg Se/L.

Radioactive Sample Analyses

Water samples were analyzed for ^{75}Se and ^{203}Hg according to Hesslein et al. (1980). The samples were first filtered (GFC-Whatman, then 0.45 μm Millipore®) to obtain the quantity of label associated with the particulate fraction. The filtrate was then sequentially passed through charcoal, anion, and cation exchange columns (Dowex 1, ×8

100–200 mesh and 50W, ×12 100–200 mesh) to separate the fractions of the two labels which were associated with organic, negatively and positively charged material, respectively. Some of the dissolved ^{203}Hg passed through all of the columns and was precipitated as ^{203}Hg-sulfide after a sulfuric acid–persulfate digestion. None of the ^{75}Se passed through the exchange columns (P. Santschi, Lamont-Doherty Geological Observatory, 1977 personal communication). Movement of ^{75}Se and ^{203}Hg out of the water column was computed on a halftime basis which is defined as the time required for half of the label to leave the water column.

Bioaccumulation rates of ^{75}Se and ^{203}Hg were followed by routinely sampling several organisms contained in the experimental tubes. These rates were compared to the control tubes to assess the ecosystem effects of the tube treatments. Pearl dace (*Semotilus margarita*) were released into the tubes in two batches at 3 and 39 d after day zero. The fish stocking density was about 50 g/ha (1 ha = 10 000 m^2) which ensured ample natural food in the tubes. Individually caged crayfish (*Orconectes virilis*) were maintained in the tubes in contact with the sediments. An excess of unlabeled white sucker flesh was provided as food. Bioaccumulation of the labels by haptobenthic communities was followed using 1.5 m × 3.3 cm strips of the polyethylene tubing material which had been colonized in the lake for 1 m prior to introduction into the tubes. On each sampling date, one strip from each tube was cut into sections and assayed for ^{75}Se and ^{203}Hg bioaccumulation. Subsequently, the attached material from each section was dried, removed by scraping, and weighed.

Radioactive samples were assayed by gamma spectroscopy using a germanium–lithium detector (Ortec) connected to a computer-based Ultima II multichannel analyzer (Ino-Tech). The samples were contained in small Petri dishes (50 mm in diameter, 9 mm in height) that provided a consistent ideal counting geometry which enabled counts per minute to be compared from sample to sample (Hesslein et al. 1980). All samples were corrected for radioactive decay by normalizing the counts to day zero. Corrections were made for the interference of ^{203}Hg counts by ^{75}Se and are expressed on a count per minute per gram basis.

Four fish and crayfish samples were counted for each sampling date. Sample means were calculated from log-transformed data. When means for a single sampling date were compared for the control and treated tubes, the range of the data frequently overlapped but the log-normal means of the treated tubes were consistently above or below the control tube. Therefore treatment effects were clearly discernible from the controls.

Mercury concentration in fish tissues was assayed by the method of Armstrong and Uthe (1971). A modification of the semiautomated flameless method of Vijan and Wood (1974, 1976) was used to determine selenium in tissue.

Results

Selenium and Mercury Geochemistry

With the exception of the bottom-sealed tube, none of the other tube treatments affected the rates of movement of ^{203}Hg out of the water column (Fig. 1). Labeled mercury left the water column at an exponential rate with halftimes ranging from 16 to 20 d (Table 2). Labeled mercury concentrations also decreased exponentially in the bottom-sealed tube but the halftime was increased in the absence of sediments to 36 d. In the case of ^{75}Se (Fig. 1), water column concentrations decreased linearly and with longer halftimes (49–56 d) than ^{203}Hg. In the absence of sediments in the bottom-sealed tube, ^{75}Se did not leave the water column at a measurable rate (Table 2, Fig. 1).

TABLE 2. Halftimes (days) of ^{75}Se and ^{203}Hg in the water columns of the enclosures.

	Control	Selenium	Bottom-sealed	Sulfate	Herbicide
Total ^{75}Se	56	49	No significant loss	47	57
% of variation explained by a linear concentration decrease	89	96	9	85	87
Total ^{203}Hg	18	16	36	20	16
% of variation explained by an exponential concentration decrease	99	92	93	86	98

Even though the presence of elevated selenium concentrations did not have a large effect on the rate of movement of ^{203}Hg out of the water column (Fig. 1), the partitioning of the label within the water column did seem to be affected (Fig. 2). In the selenium tube the percentage of ^{203}Hg in the particulate phase was consistently the largest, and the percentage remained quite stable fluctuating only by about 4% during the course of the experiment. In the control tube the percentage of ^{203}Hg in the particulate fraction varied with time, and the particulate and charcoal fraction dominated alternately (Fig. 2).

Even though the rate of removal of ^{75}Se was not affected by the presence of 0.1 mg Se/L (Fig. 1), the stable selenite had a marked effect on the partitioning of the selenite into the various fractions (Fig. 3). In the control tube most of the ^{75}Se appeared in the charcoal fraction and was probably associated with dissolved organic matter. Most of the remainder was probably present as selenite or possibly selenate since it was in the anion fraction, while only about 10% appeared as particulate. There were negligible amounts in the cation fraction. In the selenium tube only 10–15% of the ^{75}Se was associated with the charcoal fraction, suggesting that the organic matter was rapidly saturated with selenite (Fig. 3). In this case most of the remainder of the ^{75}Se was in the anion fraction. During the course of the experiment it was calculated that 0.055 mg/L Se left the water column primarily from the anion fraction (Fig. 1 and 3). This increased the sediment concentrations in the top 2 cm from the background levels of 0.3 μg/g to about 15 μg/g.

After 100 d >90% of the ^{75}Se and ^{203}Hg in the sediments of the tubes had not penetrated past a depth of 3 cm (Rudd et al. 1979).

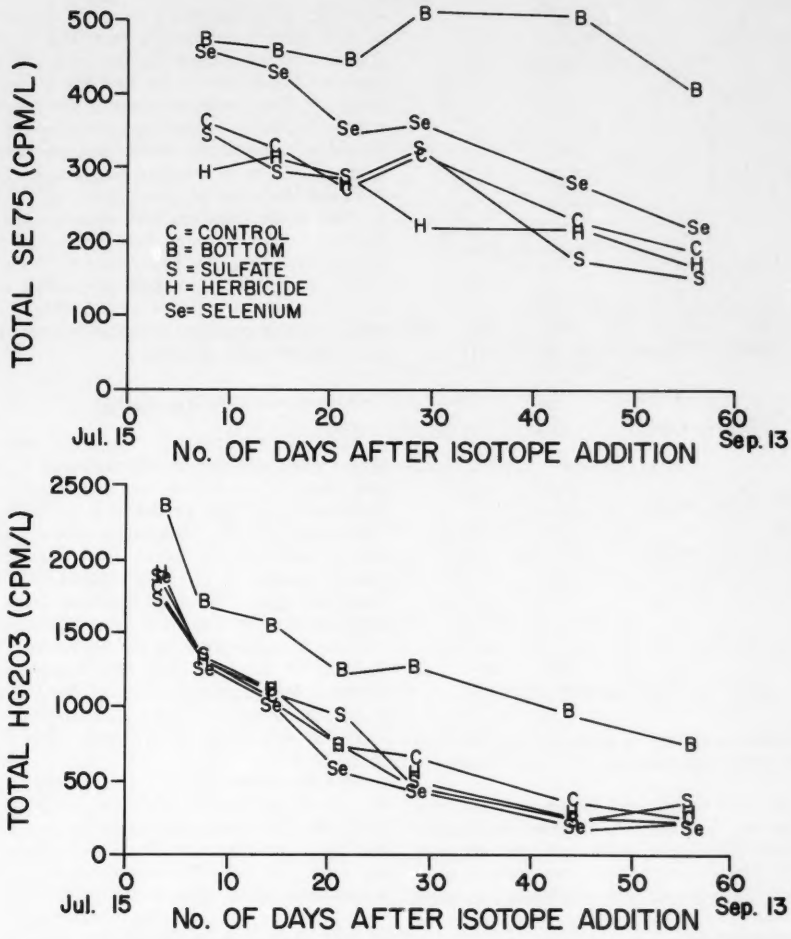

FIG. 1. Concentrations with time of total ^{75}Se in the water column of the control, sulfate, herbicide, selenium, and bottom-sealed tubes (*upper panel*). Concentrations with time of ^{203}Hg in the water column of the control, sulfate, herbicide, selenium, and bottom-sealed tubes (*lower panel*).

SELENIUM BIOACCUMULATION

Since stable and labeled selenium were added to the selenium tube simultaneously, enabling calculation of the specific activity of the added ^{75}Se, movement of the stable selenium into aquatic biota could be computed on a $\mu g/g$ basis. During the initial days of the experiment, while selenium concentrations were still relatively high in the water column (Fig. 1), pearl dace and crayfish did not accumulate significant quantities of selenium (Fig. 4). Approximately 2 wk after spike day the animals began to accumulate selenium very rapidly. This lag time evidently was not a function of fish processes since the batch 2 pearl dace began to accumulate Se as soon as they were added to the tube (Fig. 4). Movement of selenium into fish and crayfish muscle was detected at the same time as whole body bioaccumulation was first detected. The rapidity with which selenium can be bioaccumulated was demonstrated by the batch 2 dace which accumulated selenium at a rate of $\sim 0.1\ \mu g \cdot g^{-1} \cdot d^{-1}$ reaching a level in excess of $2\ \mu g \cdot g^{-1}$ by the end of the experiment. A lag was also

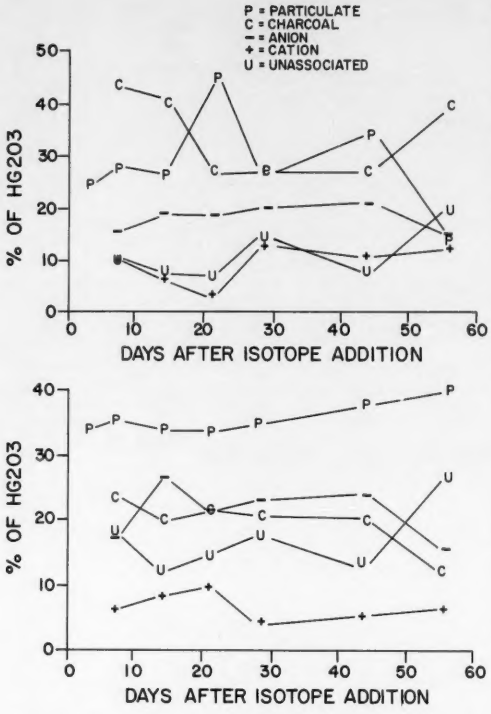

FIG. 2. Fractionation of ^{203}Hg in the water column of the control (*upper panel*) and selenium (*lower panel*) tubes.

observed in the control tube and in the herbicide tube (Fig. 5). After day 5 in batch 1, selenium bioaccumulation was consistently lower in the herbicide tube than in the control. This difference was even more marked in the batch 2 dace where ^{75}Se concentrations were usually five times lower in the herbicide tube than in the control (data not shown).

There was no lag period for selenium bioaccumulation in the bottom-sealed tube or in the sulfate tube (Fig. 5). The very high rate of ^{75}Se bioaccumulation in the bottom-sealed tube resembles the extremely high rates of ^{203}Hg bioaccumulation in this tube (J. W. M. Rudd et al. in preparation). An example of the immediate rapid selenium bioaccumulation in the sulfate tube is also shown in Fig. 5. An explanation of this may be associated with the chemical similarities of sulfur and selenium coupled with the elevated sulfate concentrations in the sulfate tube. ^{75}Se bioaccumulation by crayfish closely mimicked that by dace in all of the tubes, e.g. Fig. 6 vs. Fig. 7.

^{203}Hg Bioaccumulation in the Control and Selenium Tubes

The selenium addition appeared to decrease the rate of ^{203}Hg bioaccumulation by about half in the muscle of both batch 1 and batch 2 dace (Fig. 6). When batch 2 dace were introduced into the tubes (39 d after spike day) ~75% of the ^{203}Hg had left the water column (Fig. 1). Thus, whether most of the labeled mercury was in the water column or sediments did not change the relative magnitude of the selenium effect. Accumulation of ^{203}Hg in the dace whole body (not shown) followed the same general pattern as for the muscle, i.e. fish in the selenium tube accumulated mercury in the whole body at about half the rate of those in the control tube (Fig. 6). Movement of ^{203}Hg into whole body and abdominal muscle of crayfish was also decreased by about a factor of two (Fig. 7). A similar effect was also noted for the haptobenthos in the control and selenium tubes (Fig. 8).

Discussion

The average halftime of ^{75}Se in the water column of all the tubes exposed to sediments was 52 d. This was very close to the 47.5-d ^{75}Se halftime in Lake 224, a Canadian Shield lake (Hesslein et al. 1980), and about twice as long as that observed in tube experiments carried out on Lake 223, another Experimental Lakes Area lake (Schindler et al. 1979). Hesslein et al. (1980) suggested that ^{75}Se was removed from the water column either by deposition of particulates containing ^{75}Se or by adsorption to the sediments. The shorter halftime of the Lake 223 tube experiments may be linked to the larger proportion of the ^{75}Se that was on particulate material (35%) as compared to 20% for Lake 224 and about 15% for the Clay Lake experiments (Fig. 3).

Both the water column halftimes and the proportion of ^{75}Se on particulates were similar in the Clay Lake and Lake 224 work which suggests that the sediments had similar affinities for ^{75}Se. This is somewhat surprising since the Lake 224 sediments are sand overlain with coarse gravel while the Clay Lake tube sediments were primarily fine grey clay overlain with a thin layer of organic floc. Even though the type of underlying sediment does not seem to have a significant effect on the half time of selenium in the water column, underlying sediments were necessary for the removal of selenium from the water column. This is evident in the bottom-sealed tube where ^{75}Se concentrations remained stable throughout the experiment. Evidently the surface organic floc was not the determining factor in the removal of sediment ^{75}Se from the water since it was present in Lake 224, in the tubes with sediments, and on the plastic bottom of the bottom-sealed tube.

There appeared to be very little interaction of mercury and selenium in the water column of the tubes even though mercury and selenium have a very high chemical affinity (Frost 1972). For instance, the water column halftimes differed (Table 2) and in the bottom-sealed tube mercury left the water column exponentially while ^{75}Se concentrations remained stable (Fig. 1). In

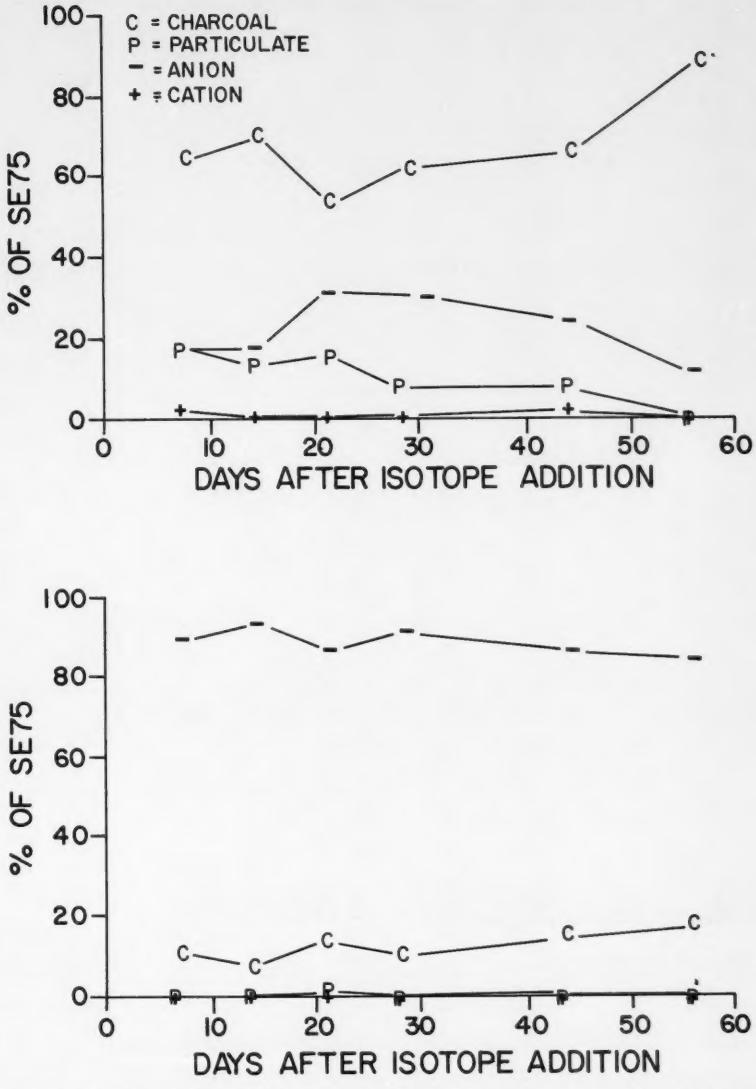

FIG. 3. Fractionation of ^{75}Se in the water column of the control (*upper panel*) and selenium (*lower panel*) tubes.

all of the other tubes movement of selenium out of the water column was linear rather than exponential as is the case of ^{203}Hg (Fig. 1). In addition, in all of the tubes except the selenium tube, the charcoal fraction, which is thought to comprise the organic fraction, contained at least 60% of the ^{75}Se in the water sample (Fig. 3). The percentage of ^{203}Hg in the charcoal fraction usually varied between 20 and 30%. A stronger affinity of ^{75}Se for the charcoal fraction as compared to ^{203}Hg was also noted by Hesslein et al. (1980).

Even though the ^{75}Se and ^{203}Hg did not move together within the water column, the elevated selenium concentration stabilized the quantity of the ^{203}Hg in the particulate fraction (Fig. 2). The periodic move-

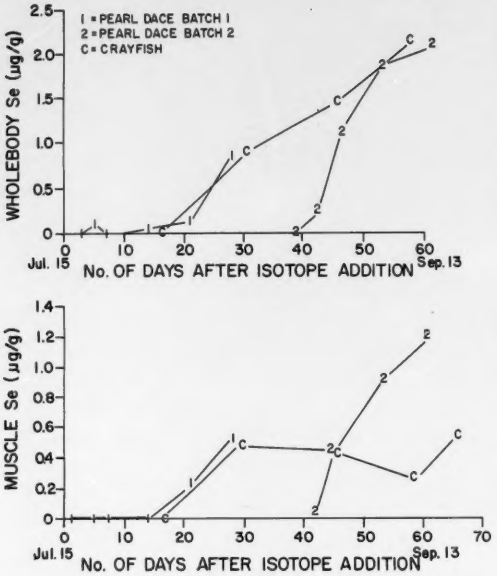

FIG. 4. Movement of stable Se into whole body (*upper panel*) and muscle (*lower panel*) of pearl dace and crayfish taken from the selenium tube.

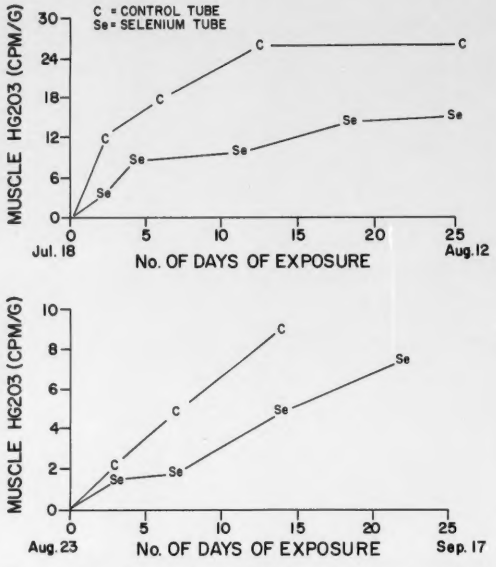

FIG. 6. Bioaccumulation of ^{203}Hg into the muscle of pearl dace in the control and selenium tubes; batch 1 (*upper panel*), batch 2 (*lower panel*).

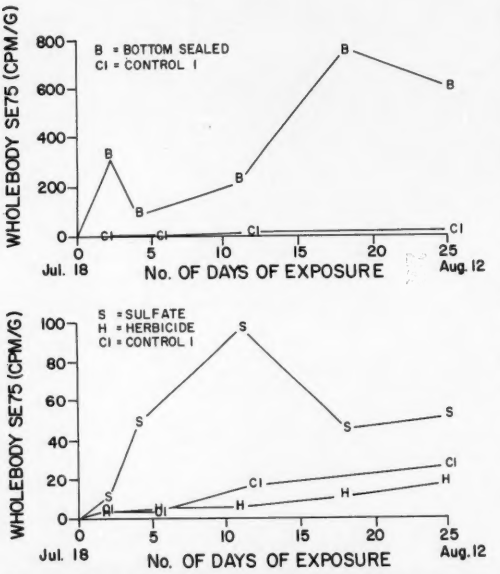

FIG. 5. Movement of ^{75}Se into batch 1 whole body pearl dace taken from the bottom-sealed, sulfate, herbicide, and control tubes.

ment of ^{203}Hg between the charcoal and particulate fractions in other tubes was eliminated in the selenium tube. Lack of movement of ^{203}Hg into phytoplankton during bloom periods in the selenium tube is probably not an explanation of this stabilization since particulate ^{203}Hg showed no correlation with fluctuations in chlorophyll concentrations or primary production potential. The elevated selenium concentration may have affected the abiotic clay component of the particulate fraction in some manner.

There are several lines of evidence which suggest that selenium was entering the biota through the food chain. The lag times for entry of selenium into fish and crayfish (Fig. 4 and 5) suggest that selenium had to enter another compartment before it was available to the fish and invertebrate. This is supported by the absence of lag time when the batch 2 dace were added to the selenium tube (Fig. 4). Similar lag times were noted in the control tube and the herbicide tube (Fig. 5). Selenium bioaccumulation by dace in the herbicide tubes was up to five times slower than in the control tube and there was also a slight lag time for the batch 2 dace. Since the herbicide interferes with the photosynthetic first stage of the food chain, these data also suggest that the food chain may be the primary route of selenium bioaccumulation. These observations agree with those of Sandholm et al. (1973). They found in laboratory aquaria that fish concentrated only small amounts of selenium directly from water. Selenium

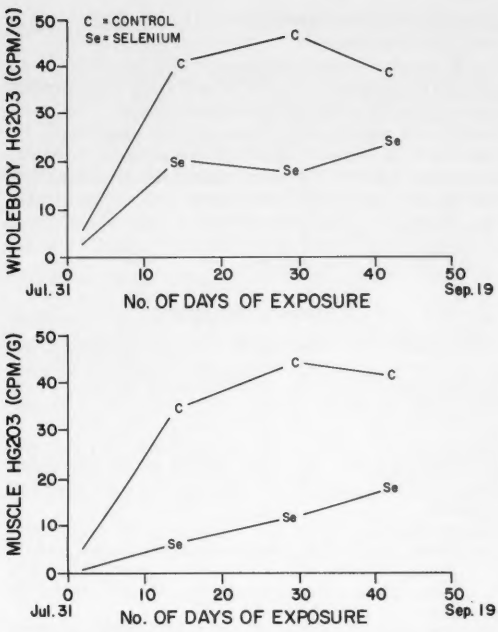

FIG. 7. Bioaccumulation of ^{203}Hg into whole body (*upper panel*) and muscle (*lower panel*) of crayfish taken from the selenium and control tubes.

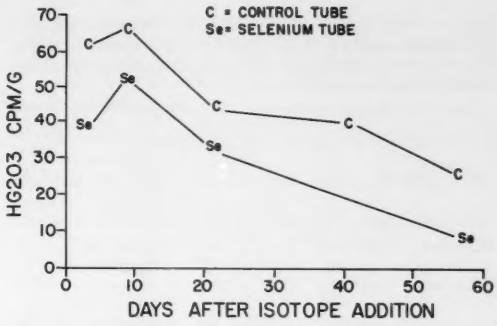

FIG. 8. Bioaccumulation of ^{203}Hg by haptobenthos in the control and selenium tubes.

bioaccumulation occurred mainly when the zooplankton food source was present.

There is also a possibility that the lag time in selenium bioaccumulation could be explained by the time required for selenite to be biologically converted into a more available form which could be readily bioaccumulated by fish directly from the water column. Experiments are currently in progress which will clarify this point.

There was no lag in ^{75}Se bioaccumulation in the bottom-sealed and sulfate tubes (Fig. 5). Suspended clay rapidly sedimented in the bottom-sealed tube unlike in the other tubes. Decreased suspended clay has been found to increase the rates of both ^{75}Se and ^{203}Hg bioaccumulation (J. W. M. Rudd et al. in preparation). The chemical similarities of sulfur and selenium may be related to the absence of a lag period at elevated sulfate concentrations.

The absence of a lag time for ^{203}Hg bioaccumulation for dace and crayfish (Fig. 6) again suggests that mercury and selenium were not moving together through the ecosystem and that bioaccumulation of ^{203}Hg directly from the water was an important route of uptake (J. W. M. Rudd et al. in preparation).

Although our experiments were not specifically designed as selenium toxicity tests we did make some general observations. At 1.0 mg/L Se in another tube we observed progressive death of young-of-the-year perch (*Perca flavescens*) beginning after 4 d of exposure and continuing throughout the remaining 3 wk of the experiment. However, there were no deaths of pearl dace or crayfish at this Se level. There were no deaths of either fish or crayfish at 0.1 mg Se/L. Clams (*Anodonta* sp.) in the 1.0 mg/L tube seemed to burrow into the clay substrate more than in the 0.1 mg/L Se tube. In the 1.0 mg/L selenium tube the top 2 cm of the clay sediments developed a gel-like consistency. This was also noted in the 0.1 mg/L tube but to a much lesser extent.

The information obtained in these experiments and the established detoxifying effect of selenium on a variety of other heavy metals (cadmium, lead, tellurium, silver, and copper; Frost 1972) as well as mercury leads to an interesting question of whether or not increasing natural selenium concentrations in freshwater systems could ameliorate heavy metal pollution problems. From our preliminary work this may be possible in the case of mercury. The mercury–selenium ratio in mercury-contaminated freshwater fish tissue could be reduced so that it would approximate that of some marine fish that contain high mercury which is nontoxic to fish consumers (e.g. Friedman et al. 1978; Ganther and Sunde 1974; Ganther et al. 1972; Ohi et al. 1976; Freeman et al. 1978; Frost 1972; Frost and Lish 1975).

Our preliminary results suggest that mercury–selenium ratios in biota of mercury-contaminated freshwater systems could be favorably adjusted (Fig. 4). Within 3 wk in the 0.1 mg Se/L tube the selenium concentrations in the batch 2 dace reached the level at which selenium would detoxify mercury present in Clay Lake fish (Potter and Matrone 1973; Ganther et al. 1972; Ganther and Sunde 1974; Chang et al. 1977). In practice it seems probable that selenium concentrations of less than the 10 μg/L drinking water standard would be required to increase selenium concentrations

TABLE 3. Atomic ratios and average 1977 selenium and mercury concentrations in Clay Lake fish and 0–6-cm sediments. Five samples per fish species and seven sediment samples from three locations were analyzed.

		Hg μg/g	Se μg/g	Atomic ratio Hg/Se
Sediment	(dry wt)	3.10±0.96	0.41±0.04	3.1
White sucker	(wet wt)	1.59±0.72	0.15±0.02	4.2
Whitefish	(wet wt)	0.95±0.39	0.21±0.01	1.9
Walleye	(wet wt)	5.67±1.65	0.23±0.03	9.8
Pike	(wet wt)	4.28±1.69	0.18±0.02	9.7

sufficiently in fish since it could be bioaccumulated gradually as they matured.

Clay Lake fish and sediment selenium and mercury concentrations are presented in Table 3. Although the sample sizes are quite small, it appears that in the cases of whitefish and white sucker, mercury and selenium were bioaccumulated in about the same molar ratios as was present in sediments. Mercury was preferentially bioaccumulated by the predatory fish (pike and walleye). This preferential mercury bioaccumulation may be the result of accumulation of mercury from other sources than food or water. For example, mercury methylation in gut contents of pike and walleye is relatively high in comparison to whitefish and sucker gut contents (J. W. M. Rudd et al. in preparation). Even though mercury–selenium ratios increased at higher levels in the food chain, selenium concentrations would probably not have to be increased proportionately if selenium was added to the lake as an amelioration agent. Mercury to selenium ratios below 1:1 were found to be effective in decreasing neurological symptoms and death by methylmercury intoxication in quail (Stoews et al. 1974; Chang et al. 1977).

A second means by which added selenium could ameliorate mercury pollution would be for it to reduce the rate of mercury bioaccumulation by aquatic biota. All of the biota tested in our experiments (pearl dace, crayfish, and the haptobenthic community) bioaccumulated ^{203}Hg at about one-half the rate of the control (Fig. 6–8). In these experiments we measured the bioaccumulation of total ^{203}Hg into fish and crayfish muscle. We have since found that this selenium treatment affects the bioaccumulation of total and methylmercury by fish and crayfish muscle in the same manner (M. A. Turner et al. in preparation).

The rapid bioaccumulation of added selenium by fish and crayfish could very likely be repeated on a whole-lake basis and should therefore be considered as a distinct possibility for ameliorating mercury and other heavy metal pollution problems. On the other hand, even though ^{203}Hg bioaccumulation was consistently reduced in fish, crayfish, and haptobenthos in the presence of elevated selenium, we are viewing these as preliminary results which require verification. This work is presently underway.

Of course, before selenium could be used as an amelioration agent, it would be necessary to investigate thoroughly the direct and indirect aspects of selenium toxicity to aquatic organisms and fish consumers, including man. Certain aspects of this work are currently underway and may provide management alternatives to mercury and other heavy metal pollution problems. Knowledge of these interactions is also important to determine the impact of atmospheric fallout of heavy metals on aquatic ecosystems.

Acknowledgments

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Necessary Components of Economic Surplus in Fisheries Economics

LEE G. ANDERSON

College of Marine Studies and Department of Economics, University of Delaware, Newark, DE 19711, USA

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There are four main types of economic surpluses that can be achieved when exploiting a fishery. They are rent to the productive nature of the fish stock, normal factor rents to inputs, consumer surplus, and what is called here, worker satisfaction bonus, WSB. The latter refers to nonmonetary benefits individuals can obtain from participating in commercial fishing and, to be complete, in other occupations as well. It is discussed frequently, but has never received any formal treatment in the literature. In this paper the logic behind WSB in the fishing industry is discussed and its implications on policy is shown. A detailed model is developed which can show how all of the components of economic surplus relate to one another and how they will vary at different levels of output.

Key words: fisheries economics, economic surplus, worker satisfaction bonus, maximum economic yield

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Dans l'exploitation d'une pêcherie, quatre types principaux de surplus économiques peuvent être atteints. Ce sont: revenu à la nature productive du stock de poissons, revenus de facteurs normaux aux inputs, surplus au consommateur et ce que nous appelons ici prime de satisfaction au travailleur, WSB («worker satisfaction bonus»). Il s'agit dans ce dernier cas de bénifices non monétaires que peuvent retirer les individus de la pêche commerciale, comme d'ailleurs, pour être complet, dans d'autres occupations. Ce facteur est souvent mentionné mais n'a jamais reçu de traitement formel dans les travaux publiés. Le présent article analyse la logique sous-tendant le WSB dans l'industrie halieutique et démontre ses implications dans l'élaboration des politiques. On y construit un modèle détaillé pouvant montrer comment tous les éléments du surplus économique sont liés entre eux et comment ils peuvent varier à divers paliers d'output.

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SEMINAL work on fisheries economics by Gordon (1953) has established that an open-access fishery will result in a suboptimal allocation of resources as far as strict economic efficiency is concerned. He demonstrated that a proper allocation will occur when the social surplus from the fishery is maximized. He assumed that price was constant and so equated profit to the fishery as net social surplus (p. 141). For policy prescription purposes, however, a more general model is necessary so that the social surplus maximand can be properly specified. In addition to profit to the fishing industry, this maximand should include at least consumer surplus and rents to all factors of production (Copes 1970, 1972; Anderson 1973). (In Copes' paper, the term producer's surplus is used. I have chosen to follow Mishan (1968) and Currie et al. (1971) and use factor rents. As the latter paper argues, there are compelling reasons to do so (p. 756–758), and in addition it will make discussion of the concept of the worker satisfaction bonus introduced below more understandable.) Nevertheless, during discussions of fisheries regulation, Gordon's maximand consisting only of industry profit is quite often used. And, perhaps more important, it is the maximand that many noneconomist fisheries researchers think economists are talking about when we say rent maximization. For example, John H. Steele, an noneconomist and Director of the Woods Hole Oceanographic Institution, commenting on the optimal level of effort in fisheries said: "The basic economic consideration is often not the biological yield but the profitability obtained by the fishery fleet . . . ," (Anon. 1978, p. 6). It is apparent that economists have still not succeeded in presenting the complete notion of economic efficiency.

It is sometimes argued, furthermore, that individuals who participate in commercial fisheries derive some benefit from the nature of the work, hereafter called worker satisfaction bonus, or WSB. At a recent conference on Limited Entry and Fisheries Management, this was expressed from the floor by a spokesperson from the fishing industry as: "Fishermen do not only work for the money. There is something else involved. If you have been a fisherman, there is no need to explain it. If you have not, it would be impossible to explain it." If it can be explained or not, if these benefits exist, then they should also be included in the maximand. In the pages that follow a model is developed that allows for joint consideration of WSB, other factor

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rents, industry profit, and consumer surplus. While a dynamic format is necessary for a complete description of the optimal utilization of fishery resources, the subject at hand can be treated in a fully adequate manner in a static model. The objectives in presenting this model are:

1) To discuss the idea of a worker satisfaction bonus which has received no formal treatment in the fisheries literature to date and yet is a topic that is often mentioned in qualitative terms during discussion of applied fisheries management. Such a formal treatment is necessary because many management agencies including the National Marine Fisheries Service and the Regional Management Councils in the United States are giving attention to these effects in determining management objectives. Also the discussion will prove useful because the share system for determining wages in fisheries makes determination of opportunity cost measurements very difficult and this difficulty is compounded by the existence of a WSB;

2) To stress again that the proper maximand to achieve economic efficiency in fisheries management is the sum of all rents and consumer surplus and to show that if the proper maximand is used, many of the differences between economic efficiency management objectives and social management objectives can be eliminated (for a detailed discussion of social objectives in fisheries management see Crutchfield (1972), Gulland (1977), Knight (1975, p. 15–23), and Orbach (1979));

3) To demonstrate the relative changes in the various components of the maximand at different critical levels of fishing activity.

To place the analysis in perspective, it should be noted that the concepts behind the analysis designed to achieve objectives (2) and (3) are certainly not new. They are a basic part of standard economic analysis, and, as pointed out above, are implicit in the pioneering work of H. Scott Gordon, which applies this analysis to open-access fisheries. However, to achieve objective 1 adequately, it was necessary to build a model which considered all components of economic surplus. It therefore appeared useful, in view of confusions (at least as perceived by this writer) concerning the application of the rent maximization principle, that a detailed reexamination of the topic was in order. It certainly will be very difficult to introduce economic efficiency principles in applied management problems if these confusions persist. It was for this reason that the second two objectives were added to the paper and why the later sections are more detailed than they need be to explain the concept and the policy implications of a worker satisfaction bonus.

The first section of the paper discusses WSB and factor rents. Profit and consumer surplus are discussed in the second section. Next all four of the components of the maximand are described for various levels of effort. The major results of the paper are discussed in the summary.

Worker Satisfaction Bonus and Normal Factor Rent

The argument for the existence of a WSB is based on the premise that employment in fishing provides satisfaction from such things as adventure, risk taking, challenges with the elements, fellowship, and ties to traditional behavior which satisfaction is presumably not available to the same degree in other types of work available to them. For example, Cicin-Sain (1979) states:

> "As psychological theory has long told us, individual behavior is motivated by many and variegated factors, such as monetary inducements, needs for association, needs for self-expression and self-actualization, status recognition, and so forth. Moreover, in an activity such as fishing, non-material incentives may play a more prominant role that they do in other occupational endeavors. Much as in academic life, work environment characteristics of freedom, independence and 'being one's own boss' which are associated with fishing may be crucial ingredients in accounting for recruitment patterns into the occupation and for high levels of work satisfaction."

In a similar manner, Orbach (1979) has written:

> " 'Recreational' fishermen may value their surroundings while fishing; they may 'subsist' on their catch in the sense that they consume it personally; they may value the right to fish as tradition, and possibly even as necessary to their physical or mental health; they also derive social status and solidarity from sharing their catch with neighbors or others in their social network; they may also gain remuneration from the sale of their catch. What is often overlooked is that 'commercial' fishermen value their fishing rights for exactly the same reasons, albeit in somewhat different mixes . . . our inattention to the *range* of benefits derived by specific users — for example, *aesthetic* benefits derived by *commercial* users — has been a significant contributor to our confusion in addressing management issues."

Poggie and Pollnac (1978) and Pollnac and Poggie (1978a, b) have gone into considerable detail to describe the components of what I have called here, worker satisfaction bonus, but it would go beyond the scope of this paper to review their work here.

Two important points are in order. First, it is certainly possible that a WSB may exist in other industries. Second, it is also possible that WSB in fisheries is negative. That is, there may be negative utilities associated with fishing. A recent discussion of the subject by a firsthand observer indicated at best that fishermen have a love–hate relationship with their work (Boeri and Gibson 1976, Chap. 3). A proper analysis of WSB must be able to consider both of these points. This can

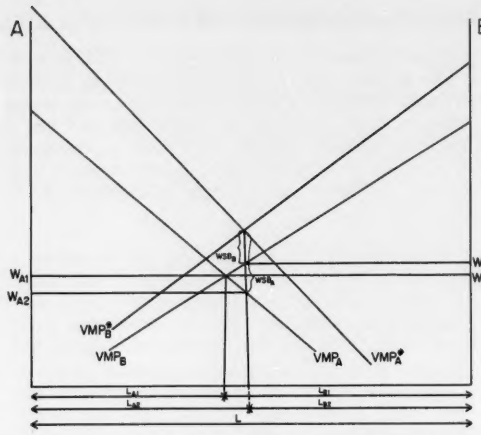

FIG. 1. A geometrical model for determining the allocation of labor between two industries. The VMP* curves represent the value of the marginal product of the last worker in a respective industry including the worker satisfaction bonus. Note that with a WSB there will be a different money wage in each industry at the equilibrium point.

be done fairly straightforwardly with a simplified general equilibrium framework. The presentation of this framework will comprise the first half of this section. For the analysis of WSB to be useful for the general purposes of this paper, however, it will be necessary to discuss WSB in a partial equilibrium framework. Such a model comprises the second half of this section. When moving from the general to the partial analysis, it will be assumed that WSB exists only in fisheries and will always be positive. This will be done to allow for graphical and exposition simplification. In drawing conclusions and making policy recommendations from this partial equilibrium analysis, every attempt will be made to take direct cognizance of the effects these restrictive assumptions will have.

The basics of a simple general equilibrium model that can explain WSB and its effect on relative wages is presented in Fig. 1. Assume that there is only one input, labor, and two industries in the economy. To make the necessary points in a "neutral" manner, it will be assumed for the present that neither of the industries is fisheries. The length of the horizontal axis represents the total amount of labor that is available. The object of the analysis is to describe the allocation of labor among the two industries. The value of marginal product for labor in the A industry, which is the price of output times the marginal product of labor (for more detail see any basic economics text) is measured from the lefthand axis. Similarly the value of the marginal product of labor in the B industry is plotted from the righthand axis. These curves are labeled VMP_A and VMP_B, respectively. Ignoring for a minute the other curves in the diagram, which have to do with worker satisfaction bonus, it can be said that an optimal allocation of labor between the two industries will occur at the intersection of these two curves. That is, L_{A1} should be allocated to industry A and L_{B1} should be allocated to the B industry. At this point the wage rate in both industries will be equal as denoted by W_{A1} and W_{A2}, respectively. This is an optimal allocation because the value of the marginal product of the last unit of labor allocated to industry A equals the value of the marginal product of the last unit of labor allocated to industry B. Any reallocation of labor from one industry to the other will result in a net decrease in total value of output produced. This is the standard competitive result and as long as there is competition between producers and in the labor market, this equilibrium will be achieved.

The existence of a worker satisfaction bonus can be inserted into this model in the following manner. A positive worker satisfaction bonus can be looked at as an output or a service that is produced in the process of working in a particular industry, but which flows directly to the individual worker. In this case, the true value of the marginal product for labor in any particular industry is the value of the extra production of the output plus the monetary equivalent of the worker satisfaction bonus for the marginal worker engaged in that industry. These values are represented as the second set of curves in Fig 1 labeled VMP_A* and VMP_B*. The difference between VMP_A* and VMP_A and between VMP_B* and VMP_B represents the worker satisfaction bonus received by the last worker hired in that industry. Recognizing that this may decrease as more and more workers get into the industry (presumably because different people may derive different amounts of satisfaction from working in a particular industry, and all else equal, those that derive high satisfaction will be the first ones to enter a particular industry), these curves are drawn approaching one another as employment in the given industry expands. The reason for the decrease in the value of worker satisfaction bonus has nothing to do with the productivity in the industry necessarily, but with the range of tastes associated with the particular workers. As drawn in Fig. 1, the worker satisfaction bonus associated with work in industry A is larger than the WSB associated with work in industry B, but nonetheless both have a worker satisfaction bonus.

If these worker satisfaction bonuses do exist, the competitive market equilibrium will occur at the intersection of the second set of curves. At this equilibrium there will be L_{A2} units of labor allocated to the A industry and L_{B2} units of labor allocated to the B industry. At this point, the wage rate in each industry will be equal to the traditional value of the marginal product of labor in each industry. That is the wage rate will be W_{A2} in the A industry and W_{B2} in the B industry. The amount of worker satisfaction bonus for

the marginal worker in each industry is noted in the diagram. It is the difference between the VMP_A^* (VMP_B^*) and the VMP_A (VMP_B) curves. Note that the WSB in the A industry is greater than the WSB in the B industry. Accordingly, the monetary wage rate in the A industry is lower than the monetary wage rate in the B industry. The net remuneration, which consists of the money wage rate and the WSB, received by the workers in both industries are equal, however. The fact that the real wage rate will be equal in both industries is important. It explains why there can be a competitive equilibrium in the presence of different monetary wage rates in various industries. Workers will compare what they are receiving in terms of wage rates and in terms of worker satisfaction bonuses, and if the package offered by one industry is greater than that offered by another, there will be a tendency to move to where the net gains from employment are higher. These movements will continue until the packages offered in both industries are the same (i.e. the movement will continue until the intersection point between VMP_A^* and VMP_B^* is achieved. This equilibrium can be called a socially optimal allocation of labor because the true value of the total output in society (including output produced for sale and WSB) is maximized and any reallocation of labor would reduce it.

Note that in comparison to the situation with no WSB in either industry, there has been a shift in labor from industry B to industry A and the monetary wage has gone down in industry A but gone up in industry B. This is because as drawn, the WSB is greater in industry A than in industry B. It can be concluded from the above that in a perfectly competitive economy with no market failures (specifically with no common property or open-access resources), the existence of a worker satisfaction bonus causes no problems as far as allocation of labor or other inputs is concerned. As long as people actually do perceive a worker satisfaction bonus, and make their employment decision accordingly, an optimal allocation of inputs will be achieved. The graphical analysis presented above just allows a simple way of explaining why monetary wage differentials can persist in an equilibrium situation. As a final point note that although the worker satisfaction bonuses used above were all assumed to be positive, a negative worker satisfaction bonus could easily be introduced. In such a case the VMP* curves would be below the VMP curves.

If the existence of a worker satisfaction bonus just allows for an explanation of different monetary wage rates for similar types of labor, the question of its importance in fisheries economics naturally arises. An open-access fishery will lead to a suboptimal allocation of resources (Anderson 1977, Chap. 3). On pure economic efficiency grounds, the goal of fisheries regulation is to cause a reallocation of inputs such that the value of goods and services produced in the economy is increased. To achieve this, it is necessary to know the opportunity cost of labor and other inputs. As the

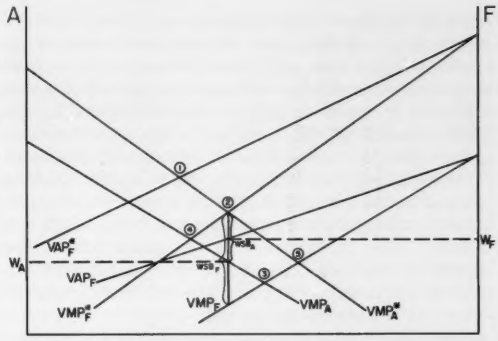

FIG. 2. A geometrical model for determining the optimal allocation of labor between a fishery and another industry. The open-access equilibrium will occur at point 1 because that is where the real net wage is equal for the two. The optimal allocation will occur at point 2 because at that point the net marginal value of production is equal in both.

above analysis has shown, in the presence of WSBs, the money wage can be an incorrect measure of the opportunity cost of labor. The importance of this will be more fully explained below.

To begin, it is necessary to show how the above analysis must be modified to consider the open-access problem in fisheries. Figure 2 is similar to Fig. 1 except that one of the industries will be a fishing industry. The A industry, whose curves are measured from the lefthand vertical axis, will be assumed to be a normal competitive industry while the F or fishing industry, whose curves are measured from the righthand vertical axis, suffers from an open-access market failure in that no one entity owns the fish stock or can otherwise control its exploitation. Because of the open-access nature of the fishing industry, it is necessary to include the value of the average product curve as well as the value of the marginal product curve. This is because, as has been demonstrated many times before, the return to fishing effort (which is the wage to fishing labor in this simple model) is the average return to the fishery (Gordon 1953; Anderson 1977). The new curves in Fig. 2 are the VAP_F curve, which measures the value of the average product (or the value of the catch per unit of effort) in the fishing industry, and the VAP_F^* curve, which is the sum of the value of the average product to fishing and the monetary valuation of the WSB to the last worker hired in fisheries.

Using this diagram it is possible to explain the difference between the open-access equilibrium level of effort, or in this case labor, and the maximum economic yield level of labor applied to the fishery. The analysis is essentially the same as that in the traditional Gordon model although it may appear somewhat different because of the two-industry approach. Assuming that workers in both the fishing and the other industry per-

ceive the worker satisfaction bonus as indicated on the graph, they will make their employment decisions accordingly. The open-access equilibrium will occur at the distribution of labor between the two industries as indicated by point 1, which is the intersection of the VMP_A* and the AMP_F* curves. At this point the value of the average product of labor in the fishing industry plus the marginal worker satisfaction bonus from fishing is equal to the value of the marginal product in the A industry plus the worker satisfaction bonus in the A industry. That is, the net real wage (the sum of the monetary wage and the WSB) is equal in both industries. Since this is the case, there will be no incentive for workers to change industries.

The optimal allocation of labor between these two industries, however, occurs at point 2 which is the intersection of VMP_F* and the VMP_A* curves. Note that this is the point of the natural equilibrium in the situation where there is no common property industry as was indicated in Fig. 1. At this point the true value of the marginal product of labor in both industries is equal. This includes the sum of the value of the marginal product and the worker satisfaction bonus. To see why point 2 represents a superior allocation of labor than does point 1, note that at the allocation at point 1, the VMP_A* is greater than VMP_F*. This means that the last worker employed in the A industry is producing a higher value of output than is the last worker in the fishing industry. The value of output in both cases represents the value of the output produced for sale plus the value of the WSB to that worker. Therefore by switching a worker from the fishing industry to the A industry, there will be an increase in the value of goods and services produced. That is, the benefits gained from such a switch (the real value of output obtained in the A industry) are greater than the opportunity cost (the real value of output foregone in the fishing industry). Note that the optimal utilization of labor results in a smaller allocation to the fishing industry than occurs under the open-access situation. The amount of the worker satisfaction bonuses to both industries at the optimal point as well as the monetary wage rates are indicated in the diagram. The reason the fishery has a total net wage higher than the other industry is that there is a rent earned from the fish stock.

The standard policy implication of this type of analysis is that there should be a reallocation of labor from the fishing industry to the other industry. The purpose for introducing this analysis, however, is to show that practical applications of this policy necessitate being able to obtain a correct estimate of the WSB in both industries. If the estimates are made incorrectly, the actual policy recommendation will be incorrect. As is often the case, the type of analysis that is useful for demonstrating one point is often not as useful in discussing others. So it is with the above two-industry analysis. It shows very clearly how worker satisfaction bonus in the fishing industry and another industry can be directly considered in determining the maximum economic yield of the fishery. However, because it focuses on only two industries, it necessarily produces the result that a change in the allocation of labor from industry to industry will result in a change in the wage rate in each. In practical applications, unless the reallocation under consideration is quite significant, switches can be made without affecting the relative wage rates. This is because the fishing industry is quite small relative to all other industries. It is possible, however, to make qualitative conclusions concerning potential errors from ignoring WSB in fishing or other types of work from this simple diagram.

For example, if WSB does exist in both industries but its presence is ignored in the nonfishing industry, the apparent optimal allocation of labor will occur at the intersection of the VMP_F* and the VMP_A curves at point 4 (Fig. 2). Since point 4 is to the left of point 2, this will call for a larger allocation of labor to the fishing industry than is actually justified. On the other hand if in attempting to determine the optimal allocation of labor the existence of a WSB is ignored in the fishing industry but considered in the other industry, the apparent optimal point is at the intersection of the VMP_F and the VMP_A* curves at point 5. Since point 5 is to the right of point 2, this will mean less labor will be allocated to the fishery than is optimal. The final alternative is that the existence of WSB is ignored in both industries. In this case, the apparent optimal point will be at the intersection of the VMP_F and the VMP_A curves at point 3. As drawn here point 3 is to the right of point 2, and so again using this as a policy guide will result in underallocation of labor to the fishery. However, if the curves were drawn such that the WSB for industry A were greater than that for fishing, intersection 3 would be to the left of point 2 and the opposite conclusion would hold.

The main conclusion from this is that if in fact WSBs are a significant element in fishing and other occupations, then proper policy cannot be developed without directly considering both of them. This is pointed out because in most instances where the argument for the existence of a WSB in fisheries is made, the implicit conclusion would be that the maximum economic yield level of effort would not be as low as would otherwise be the case. As can be seen from the above, this is only true when there is no WSB in other industries or when the WSB is relatively larger in fishing than in other industries. For the remainder of this paper I am going to assume that WSB does not exist in other industries. The purpose of the remainder of the paper is to show how the worker satisfaction bonuses can be tied into the discussion of other types of rents to a fishery. Such a presentation would be very awkward without the above simplifying assumption. It should be stressed, however, that the conclusion that the optimal level of effort to the fishery is higher in the presence of a WSB to fishing is a direct result of this assumption.

In a partial equilibrium analysis, if WSB does exist

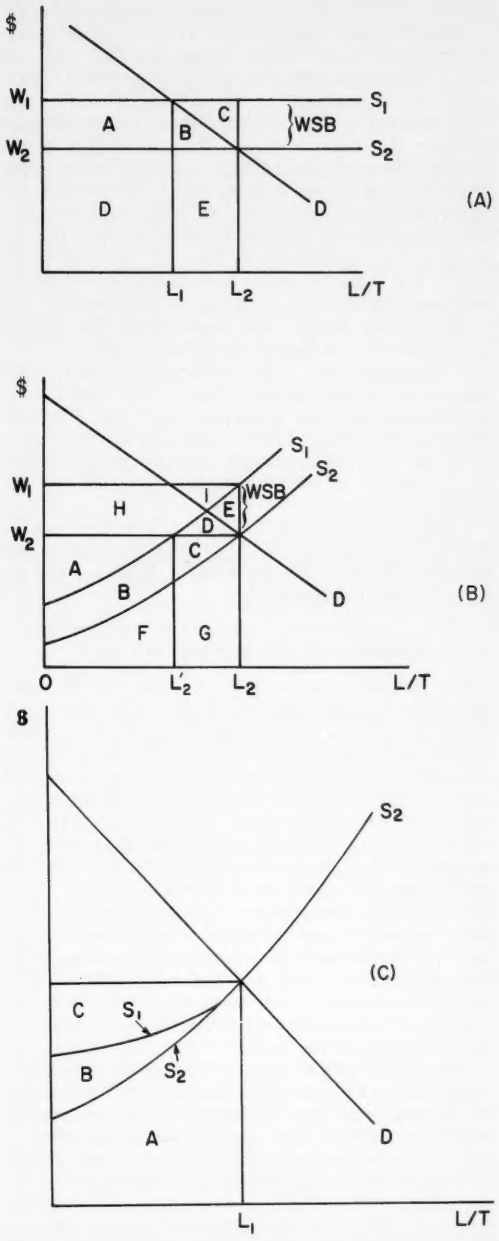

FIG. 3. A partial equilibrium analysis of WSB. Figure 3A demonstrates the case where the labor demanded for fishing is small relative to demand from other industries for this type of labor. Figure 3B is the more general case, while Fig. 3C depicts the case when WSB exists for only a portion of the labor supply.

in fishing, and all else equal, individuals in the labor pool from which fisheries draw will be willing to accept lower wages for fishing than they would in otherwise similar employment. Consider the case where labor demanded for fishing is very small relative to other demands of this type of labor. If W_1 in Fig. 3A is the market wage rate, then it would normally be the supply curve of labor for fishing, since it measures the opportunity wage rate an individual can earn elsewhere. All else equal, this is the wage that must be paid to attract a laborer to fishing.

Given the existence of a WSB, the actual supply curve to the fishery will be equal to W_2 where the difference between the two curves is the per unit monetary evaluation of WSB. Viewed in this way WSB is the premium that would be necessary to induce the fishermen to change occupations. For simplicity, the WSB will be invariant at different levels of labor, although this need not be the case. From the graph it can be seen that the fishing industry will be able to attract more workers than they would without a WSB. The total amount of worker satisfaction bonus generated is equal to areas A, B, and C. Notice that in this case the WSB does not take the form of a monetary payment to the fisherman. He just receives the value of the service provided to him. The employer, however, does receive a monetary payment in the form of a wage subsidy. To look at it another way, he is able to sell at full value the services that are provided by employment in his firm.

As far as the individual worker is concerned, the opportunity cost of employment in fishing is a wage rate of W_1. This cost is met by the wage rate of W_2 plus the WSB. Because of the positive valued service provided to the working fisherman, the net social opportunity cost of employing him in the fishery is W_2. Goods and services valued at W_1 are lost when he is employed, but $(W_1 - W_2)$ is regained through the WSB.

The significance of this difference between the two wages is quite important for regulation. Since fishermen work for a share of the catch rather than a wage, it is sometimes difficult to determine what the actual implied wage rate is. Presumably the agreed upon share system would reflect the existence of a WSB, and the percentage share to the captain and crew would be lower than it otherwise would be. That is, without a WSB, fishermen would presumably bargain for a system where their money wage rate was no lower than W_1, while with a WSB they would settle for a system giving a money wage equal to W_2. It is often suggested that to overcome the difficulty of obtaining a proper measure of opportunity cost for labor from vessel cost data, representative wages from comparable shoreside employment be used. If there is a WSB, the estimated opportunity cost wage will be W_1 when in fact it is actually W_2. As has been pointed out above and will

be discussed in more detail below, this will lead to economically inefficient policy recommendations. This is one reason why WSB should be given special attention in fisheries.

Figure 3B depicts the more general case. The curve labeled S_2 is the effective supply curve of fisherman; it represents the amount of labor that will be available in fisheries at the various wages in the long run (i.e. after movement of labor from industry to industry due to wage changes have ceased). Given the existence of WSB, then at each level of employment on the horizontal axis, there will be a wage rate for jobs similar to fishing which will be higher than the prevailing fishing wage by an amount equal to WSB. This is labeled S_1.

The area between S_2 and the price line at any level of employment is factor rents being earned by fishermen. But it is useful to break these rents into two categories. The distance between the two supply curves represents rents due to the excess payment above the minimum to keep the individual in the fishing occupation as opposed to the alternative occupation. The remainder of the rent is due to the excess payments above those necessary to keep the individual employed as opposed to engaging in leisure. The former is what we have called WSB, and for purposes here we will call the latter normal factor rent.

Equilibrium will occur at a money wage rate of W_2 and an employment level of L_2. The money wage for otherwise similar employment will be W_1. Both types of rent are being earned by the fishing labor force. Up to the level of employment where L_2' is the marginal worker, workers would be earning normal factor rents even if they were employed in a nonfishery job. The total amount of this rent earned at this level of employment is equal to A. In addition, they receive a WSB from fishing equal to area B. For marginal workers between L_2' and L_2, there is actually a negative normal factor rent because the wage rate is below what is necessary to keep them in the alternative employment as opposed to leisure. However, up to employment level L_2, the positive WSB per worker is greater than the negative normal factor rent. Therefore, individuals will continue to enter the fishery. At the equilibrium level of employment, L_2, total normal factor rent is equal to areas A − D − E, while the WSB is equal to areas B + C + D + E. The net rents to labor are equal to A + B + C.

In this case the real opportunity cost of using the equilibrium amount of labor is equal to areas F + G. Even if fishermen did work for a wage instead of a share of the catch, the market wage for fishermen, W_2, would overestimate opportunity cost, and using W_1, the wage rate for alternative employment, would give an even higher overestimate. What is necessary to estimate opportunity cost properly is an estimation of the supply curve for labor to the fishery. And this must entail a study of fishermen per se, not just comparable labor in general. Using the latter frame of reference will lead to an estimation of the S_1 supply curve rather than S_2, the effective supply curve of labor to fisheries.

Note that here again there is a subsidy, so to speak, to employers in the fishing industry in that they can pay a wage lower than for other comparable employment. The unit amount of this subsidy is equal to the WSB received by the marginal worker, i.e. the distance between the two curves at the equilibrium point. Whether the employer is able to capture all of the value provided by employment on his vessel depends upon the relationship between the value of WSB to the marginal worker and that of the intramarginal worker. The extreme case is represented in Fig. 3C, where it is assumed that there are only a limited number of people who obtain a WSB from fishing. In this case the two supply curves merge. As drawn here, the market equilibrium occurs beyond the point of merger, at L_1. The opportunity cost of labor is equal to area A while total rents to labor equals areas B + C. The former is WSB and the latter is normal factor rent. The employer in the fishing industry must pay the same wage as in the relevant alternative occupations and hence receives no subsidy even though there is a WSB to some intramarginal workers.

Looking ahead to the analysis to follow, it can be seen that in both Fig. 3A and 3B, as the level of employment increases, the net rents to fishermen will increase as well and vice versa.

Consumer Surplus and Industry Profit

For simplicity the analysis to follow will be static and will consider only one independent fishery. A fishery is defined as a particular fish stock and the harvesting equipment and labor that is capable of utilizing the stock. To eliminate any consideration of market failures other than those related to open access, let the number of enterprises in the fishery be large enough that no one can have a significant effect on prices of output or inputs, or the viability of the fish stock. It will be assumed that labor is the only variable input. This will allow for simultaneous graphic analysis of consumer surplus and industry profit with factor rents and WSB in the next section. In the meantime, however, an inelastic supply curve of labor with no WSB has been used. Where the assumption of one input will have significant effects on the results, it will be noted and discussed below.

Figure 4 contains three revenue or benefit curves which are the basis for the analysis of the relative sizes of consumer surplus and industry profit at various levels of fishing activity. They are the average revenue of labor curve, AR_L, the value of the marginal product of labor curve, VMP_L, and the marginal revenue product of labor curve, MRP_L. They can each be derived from the yield curve of the fishery and the demand curve for fish. (The basis for these curves is the yield curve: $F = 4L - 0.16L^2$, and the inverse demand curve: $P = 5 - 0.125F$.) By keeping our assumption of labor as the only variable input, the sustainable yield

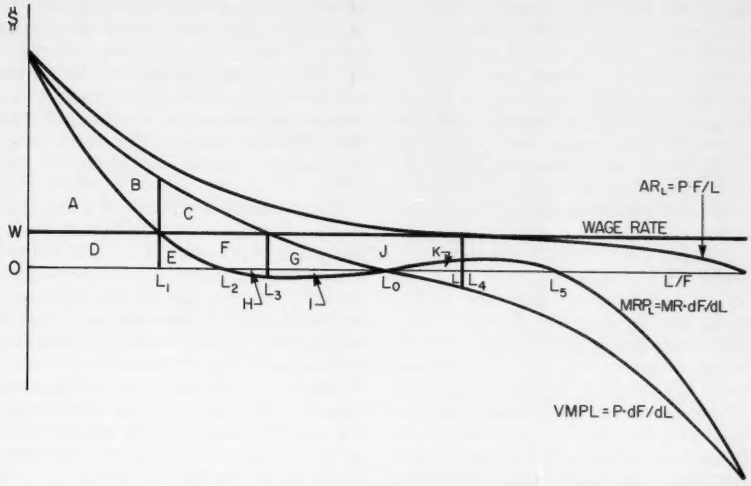

FIG. 4. A demonstration of the open-access, maximum economic yield, and the monopolistic level of output. The first occurs where the average revenue to labor (AP_L) equals the wage rate, the second where the value of the marginal product of labor (VMP_L) equals the wage rate, and the third where the marginal revenue product (MRP_L) equals the wage rate.

curve for a fish stock can be represented as

$$(1) \quad F = F(L)$$

where F reaches a maximum at L_0. The market demand curve for the fishing industry as a whole can be represented as:

$$(2) \quad P = P(F).$$

In an open-access situation, each fishing firm takes the price of fish and the average product of labor as a given. The point about price is obvious but the one about average product may require some further explanation. The yield curve is an industry-wide phenomenon. Total industry catch and hence catch per unit of labor is determined in concert by all the firms in the industry as they determine the amount of their fishing activity. Each firm may determine how much fishing it will do, but the actual amount of fish caught will depend upon the catch per unit of labor which is determined by the total amount of industry labor (Anderson 1976). The equilibrium condition for an open-access fishery is where $AR_L = W$, because AR_L for the fishery as a whole is the value of the private marginal product to the individual firm. This occurs at point L_4 in Fig. 4. As long as individual firms can have a private return greater than W they will continue to enter the fishery. Note that AR_L is decreasing for two reasons. As L increases F goes up but F/L decreases and the increase in F will cause P to fall also.

The net social benefits from utilizing the fishery can be represented by the area under the demand curve up to the given level of output minus the opportunity cost of production. In terms of labor, this can be represented as

$$(3) \quad B = \int_0^L P[F(L)]dL - wL.$$

The first-order condition for a maximum to (3) is

$$(4) \quad P\frac{dF}{dL} = w.$$

The lefthand side of equation (4) is the value of the marginal product of labor for the fishery as a whole. It is also plotted in Fig. 4. As long as the average catch per unit of effort is falling, it will lie everywhere below the AR_L curve because of the average marginal relationship. The area under this curve is equal to the sum of consumer surplus plus industry revenues. At the intersection of VMP_L with the elastic supply of labor, equation (4) will hold; therefore, given this model, L_3 is the sociably optimal level of employment, on strict economic efficiency grounds. This is often called the Maximum Economic Yield (MEY) of a fishery.

If one were concerned only with industry profit, the maximand would be

$$(5) \quad \pi = P[F(L)] \cdot F(L) - wL.$$

After simplification the first-order condition for a maximum to equation (5) is

$$(6) \quad \left(P + \frac{dP}{dF}F\right)\frac{dF}{dL} = w.$$

The lefthand side is the marginal revenue product of labor to the fishing industry. It is the third curve plotted in Fig. 4. To the left of L_0, VMP_L will be greater than MRP_L because price is always greater than MR and dF/dL is greater than zero (see equation (1) above. To the right of L_0 where dF/dL is less than zero, the price-marginal revenue relationship will cause the VMP_L to always be less in real terms than MRP_L. At the intersection of MRP_L with the supply curve, equation (6) holds; therefore operation at L_1 will generate maximum industry profits.

As drawn, the MRP_1 curve crosses the horizontal axis in three places. At L_0, dF/dL equals zero (see equation (1) above) and so both VMP_L and MRP_L are zero. Points L_2 and L_5 represent levels of labor where MR is equal to zero and so MRP_L is equal to zero here as well. Equal amounts are harvested at these points ($F(L_2) = F(L_5)$ but L_5 is beyond the point of maximum sustainable yield), and the particular amount harvested is such that the elasticity of demand at $P[F(L_2)] = P[F(L_5)]$ is equal to unity. In cases where the maximum sustainable yield is less than the amount necessary to push output into the inelastic portion of the demand curve, the MRP will not have this roller-coaster appearance, but will only intersect the horizontal axis at L_0. It will, however, always be below VMP_L to the left of L_0 and above it to the right.

The area under the MRP_L curve out to any given level of employment is the total industry revenue. When this area is below the horizontal axis it represents a negative amount of revenue. Since the area under the VMP_L curve is the sum of consumer surplus and industry revenue, and the area under the MRP_L curve is revenue, the area between these two curves represents consumer surplus. When the VMP_L curve is above the MRP_L curve, the area represents a positive amount of consumer surplus. Similarly when the VMP_L is below the MRP_L, the area between them represents a negative contribution to consumer surplus. Therefore consumer surplus reaches a maximum at the intersection of the curves.

Perhaps more explanation of the above points would be useful. Up to point L_2 where both VMP_L and MRP_L are positive, the interpretation of the areas is fairly straightforward. From L_2 to L_0 MRP_L is negative while the VMP_L is positive. Since VMP_L equals MRP_L plus consumer surplus, then the distance between the two lines is still the positive amount of consumer surplus being generated by the marginal unit of labor. The fact that part of this distance lies below the horizontal axis may confuse the fact that this represents a positive amount. From L_0 to L_5, MRP_L is positive and above the VMP_L which is negative. This means that while marginal industry revenue is positive, marginal industry revenue plus marginal consumer surplus is negative. The difference between the two lines is the decrease in consumer surplus caused by the marginal unit of labor.

The graphical interpretation of consumer surplus and profits at open access, MEY, and the monopoly position in Fig. 4 is displayed in Table 1. Consumer surplus is, as indicated above, the area between the VMP_L and the MRP_L curves. Note that at open access, consumer surplus is beyond its maximum point. Industry profit at this point is equal to zero (this is the basic conclusion of common property economics; entry will dissipate all profits), but it can be represented as the difference between the area under the MRP_L curve out to L_4 and the total wage bill. The amount in the table is the net effect after subtracting out common areas. A move from open-access (L_4) to MEY (L_3) will increase the sum of consumer surplus and profits, but a further move to the monopoly position (L_1) will reduce them.

Note that as represented here, whether a move from open access to MEY will increase consumer surplus depends upon the relative sizes of areas (G + I) and (K + L). If the latter is greater than the former, consumer surplus will increase. As is pictured here this does not appear to be the case. Operating beyond maximum sustainable yield is a necessary but not a sufficient condition for a move from open access to MEY to yield an increase in consumer surplus. But it will not be possible to predict a priori how changes in the level of effort will affect the level of consumer surplus. (The analysis here is static which in general provides results that are very general. One very important exception is the fact that MEY will always be to the left of MSY in a static model but may be on either side in a dynamic model (Anderson 1976, p. 37–42; Brown 1974). A similar argument may be made for the monopolistic situation. This makes the a priori determination of changes in consumer surplus with changes in fishing activity even more ambiguous). If the open-access equilibrium is to the left of maximum sustainable yield, (i.e. L_0), then the movement from that point to MEY will always result in a decrease in consumer surplus.

It should be remembered we are talking about consumer surplus for fish only. Other consumption goods

TABLE 1. Values associated with consumer surplus and industry profit at different levels of employment in Fig. 4.

	CS	Profits	Sum
Open access L_4	B+C+F+G+H+I−K−L	A−F−G−H−J−I=0	A+B+C−J−K−L
MEY L_3	B+C+F+H	A−F−H	A+B+C
Monopoly L_1	B	A	A+B

are not directly considered. However, barring other market failures, consumers in general do benefit from a movement from open access to MEY because as a result of the transfer of resources, the value of the fish foregone, as represented by the VMP_L curve, is less than the value of the increase in production of other goods in the economy, as measured by the wage rate for labor.

Industry profits increase with moves from open access to MEY and from MEY to the monopoly position. The profit earned at the MEY position is what is referred to as the rent from the efficiently managed fish stock. This is the rent that Gordon was referring to when he indicated that optimal utilization of resources would occur if rent were maximized. The increase in profit that occurs with the move from MEY to the monopoly position is a pure monopoly profit that results from giving market power to a single producer of fish. It can only be achieved, however, with a concurrent decrease in total economic surplus. This is the social loss of monopoly.

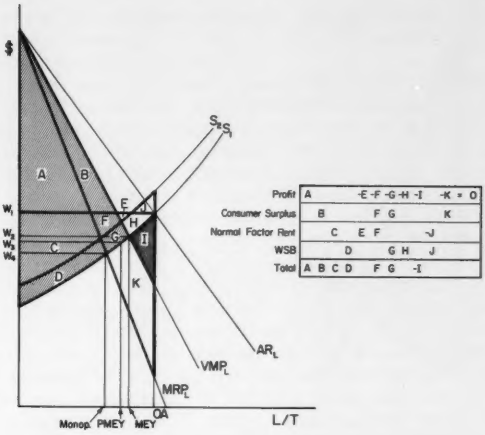

FIG. 5. Economic surplus at the open-access equilibrium yield. Open access occurs where the average revenue of labor, AR_L, intersects the effective supply curve of labor, S_1.

General Analysis

In this section the above analyses will be combined in a series of similar diagrams to show the makeup and distribution of the various types of surpluses at different levels of employment. The curves labeled S_1 will be the effective supply curve of labor to the fishing industry given an assumed positive WSB to fishing and no WSB in other types of work. The curves labeled S_2 represent the supply curve of labor for apparently similar work in other industries. To keep the graphical analysis as simple as possible, the three revenue curves from Fig. 4 will be drawn as straight lines and it will be assumed that the open-access equilibrium is below the level of effort which obtains maximum sustainable yield. This does not change the basic interpretation of the makeup and distribution of rents, with the exception of consumer surplus but the problems regarding it have already been discussed. All of the other surpluses can be defined in terms of curves that remain above the horizontal axis and whose general shapes do not change according to whether the VMP_L and the MRP_L are above or below the horizontal axis. Therefore, the interpretation of these surpluses is not affected. The general conclusions will take into account operation beyond MSY.

The fact that this model assumes that labor is the only variable input allows for a simple graphical interpretation of industry profits utilizing the MRP_L curve. When there is more than one variable input, the areas labeled profit here are actually industry revenue net of labor payments but still including any payments to other factors of production. Some of those payments may include factor rents. Along the same line, the concept of normal factor rent is restrictive in the current model; it only refers to quasi-rents to labor that are different than WSB. The concept used by Copes (1972) is more general and takes into account efficiencies in the production of fishing effort.

Figures 5 through 8 form the basis of the analysis of this section. Each depicts the three revenue curves and the two supply curves discussed above, and each describes the makeup and the size of the total social surplus achieved at a specific level of employment. In each case it is the area between the VMP_L curve and the actual supply curve for the fishery out to the appropriate amount of L.

Figure 5 depicts the open-access situation. The total social surplus achieved by society is the hatched area *minus* the shaded area. The former is where VMP_L is above the supply curve while the latter is where the reverse is true. The breakdown of this total into the four types of economic surplus is indicated in the table next to the graph. As indicated above, the industry profit will be zero at this point, but it is useful to express it as the difference of the area under the MRP_L curve and the wage bill. Note that much of the negative part of the industry profit is cancelled out by consumer surplus, and that the rest, except area I, is cancelled by the net effect of normal factor rent and WSB. Area I remains a negative element in net social welfare.

One must be very careful with regard to the use of the term "cancel out" in referring to the breakdown of the various types of surplus, however. For instance, one may be tempted to say that the consumer surplus represented by area K is possible only because there is an equal but negative effect on industry profits. Or more precisely, that in order to expand production to obtain the extra consumer surplus, there must be an increase in costs which means a decrease in the value of goods and services produced elsewhere equal to this

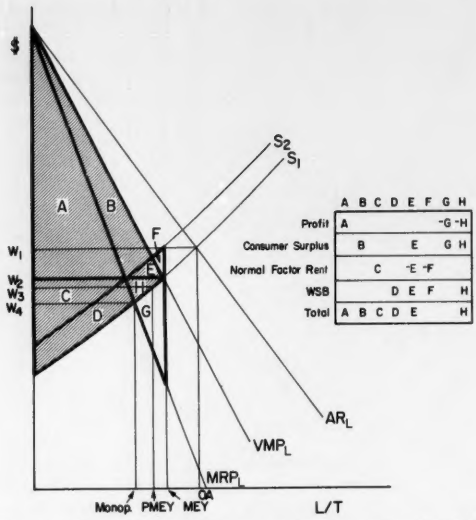

FIG. 6. Economic surplus at maximum economic yield. MEY occurs where the value of the marginal product of labor, VMP_L, intersects the effective supply curve of labor, S_1.

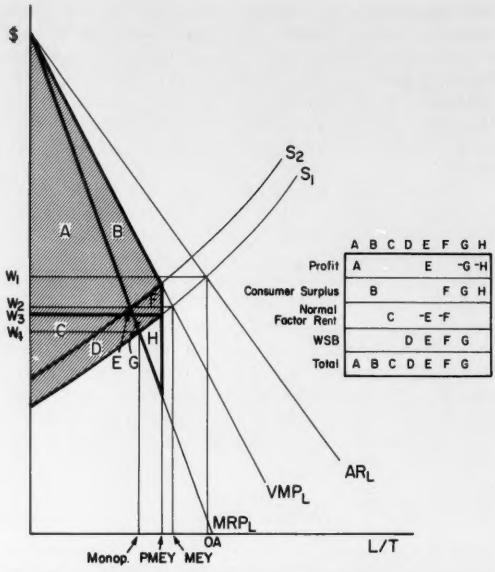

FIG. 7. Economic surplus at pseudomaximum economic yield. PMEY occurs where value of the marginal product of labor, VMP_L, intersects the supply curve for similar workers, S_2.

amount of positive consumer surplus. In actuality, however, there is no transfer from one type of surplus to another. All the graph is telling us is that in open access there are equal but opposite valued amounts of these types of surpluses. That this latter interpretation is correct is more easily seen when the same area represents a part of three different types of rent. Consider area G. Is the positive amount of consumer surplus cancelled out by the increase in cost (as is the case with area K) which leaves a WSB equal to G? Or is the decrease in industry profits due to the fact that the industry must pay a higher wage than the reservation price of intramarginal workers as appears to be the case with area H? Is it correct to say the transfer from industry to worker cancels out and leaves a positive amount of consumer surplus equal to G as a net benefit? A similar set of questions can be asked concerning area F except that there is a normal factor rent rather than a WSB. Looked at individually, both explanations sound plausible. The main thing, however, is that the algebraic sum of each is positive and enters into net social benefits.

Figure 6 depicts the MEY situation where the VMP_L curve intersects the supply curve of fishermen. The negative segment of total social surplus in Fig. 5 is no longer applicable, and it is this savings that represents the net economic benefits of moving from open access to MEY. Note that in this case all of the negative part of profits is "cancelled out" by parts of consumer surplus.

Figure 7 depicts the situation that would result from calculating MEY using the wage available in similar employment as the opportunity cost of labor. This occurs where the VMP_L curve intersects the S_2 curve. This output level is called the pseudomaximum economic yield (PMEY). Because of the supposed higher costs, the derived optimal level of employment is below that at MEY and total social surplus decreases. Again at this point the negative part of profit is cancelled out by parts of consumer surplus and areas E, F, and G are involved in a three-way cancellation.

If there is a WSB in other industries that is greater than the one earned in fisheries, then, as indicated in the previous section, using the money wage for other labor as an estimate of the opportunity cost of labor in fishing will result in a pseudomaximum economic yield to the right of MEY. When graphed it would be similar to the open-access situation in Fig. 6.

Figure 8 depicts the case which would exist if there were a single owner of the fishery. This occurs at the intersection of the MRP_L curve and the S_2 supply curve. Here there is no negative part of profit (indeed industry profit is at its maximum) and there are again areas which are common to three or more types of surplus.

Although the demarcation of areas from graph to graph are seldom directly comparable (this would involve a horrendous notation problem), there are basic similarities. Note that in each case the net social surplus is made up of a large portion of each of the

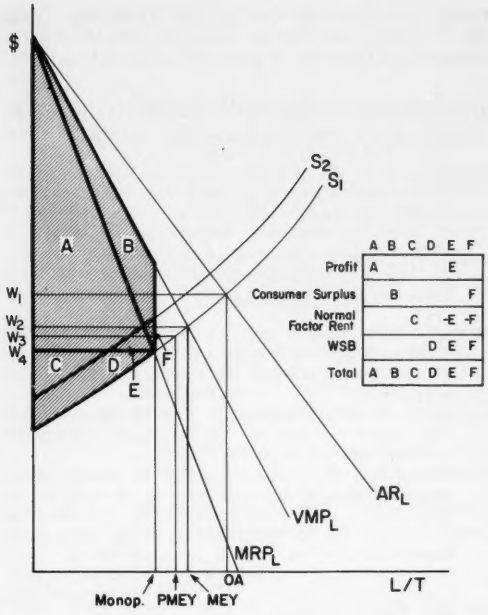

FIG. 8. Economic surplus at the monopolistic level of output. This occurs where the marginal revenue product of labor, MRP_L, intersects the effective supply curve of labor, S_1.

four types of surpluses (profits, consumer surplus, normal factor rent, and WSB) which are noted by areas A, B, C, and D, respectively, in each of the figures. There is also a portion that is composed of the net effects of three-way cancellations between areas common to three of the four types of surplus. In every case except the monopoly situation, there are some direct cancellations to two types of rent that do not show up in the net surplus at all.

TABLE 2. Qualitative changes in size of different types of surpluses with movements from one level to another.

From/to	MEY	PMEY	Monopoly
OA	P + CS ? NFR ?(−) WSB − T +	P + CS ? NFR ?(−) WSB − T ?	P + CS ? NFR ?(−) WSB − T ?
MEY		P + CS ? NRF ?(−) WSB − T −	P + CS ? NFR ?(−) WSB − T −
PMEY			P + CS ? NFR ?(−) WSB − T −

It is also interesting to note what happens to the size of the different types of surpluses with movements from one level of employment to another (Table 2). A move from open access to any of the other levels of effort will always increase industry profits. Indeed profits will increase monotonically with decreases in L until the monopolistic situation is reached. On the other hand a move from open access to any other point will always decrease the net rents of workers, the sum of WSB, and normal factor rents. The WSB will always decrease although it is possible that normal factor rent may increase in cases where the divergence between S_1 and S_2 increases rapidly. This is probably an unlikely situation, however, and in most cases the normal factor rent will decrease. Wage rates and employment levels will always decrease with decreases in L. Note that wage rates fall progressively from W_1 to W_4 if the indicated changes are made. (See Weitzman (1974) for a more rigorous model, but one which excludes WSB, that arrives at the same conclusion concerning the net effect on labor.) Although in Fig. 5 through 8 a move from open access to any other position listed will decrease consumer surplus, this is not always the case because of the problem with operations beyond MSY. Therefore the effect on consumer surplus is listed as unknown.

It is important to note that only with the move from open access to MEY can it be definitely stated that there will be an increase in net social surplus. The move to either PMEY or the monopoly situation from open access will remove the negative part of net social rent but it will also eliminate some of the positive part as well. Whether this will be a net gain depends upon the relative size of the two areas. This means that regulation procedures which use an improper measure of the opportunity cost of labor not taking into consideration the WSB or which give some monopoly power to individuals remaining in the fishery will not result in the net increase in net social benefits predicted and may in fact result in a decrease.

The effects on the breakdown ôf rents from moving from MEY to PMEY and to monopoly and from PMEY to monopoly are also listed in Table 2, but the analysis is similar and will not be discussed here.

It is interesting to note that in many cases regulation to correct the open-access inefficiency of the fishery, while leading to an increase in net social benefits, results in losses to the consumers of fishery products and to the fishery labor force, two groups closely related to the fishery. Granted there is an increase in net social surplus (which, as described above indicates a net increase in the value of goods and services produced elsewhere in the economy) that is larger than the loss in these groups and so there is potential for a net gain. This analysis does not decrease the basic logic of the economic rationale for regulating economic effi-

ciency in fisheries, but it certainly makes specific some of the distributional aspects involved which are seldom discussed. It also explains why public hearings on fisheries regulation are mostly concerned with opposition arguments since the people directly involved are usually adversely affected. On the other hand the gains are spread throughout the economy in such a way that the private benefits of arguing for regulation may well be less than private costs.

Transfer payments can ensure that some of the gains are actually received by fishermen and fish consumers, especially the former. These are possible depending upon the nature of regulation. For example, a limited entry program that grants a property right in the fishery will pass these rents back to the owners. To the extent that owners are operators, as is the case with many U.S. fishermen, or that the crews are capable of taking some of the rent in their share, there will be a net increase in the total rents received by the fishery labor force.

Summary

This paper has presented a formal analysis of the concept of a worker satisfaction bonus and its implications for management. If it exists but is ignored in developing economically efficient management plans, resource misallocations will result which will prevent the social optimum from being obtained and may even result in a decrease in welfare. Further, if worker satisfaction bonus in fishing is positive but ignored in policy recommendations, the optimal level of effort in a fishery will be underestimated. This is the conclusion that many proponents of a WSB in fisheries would like to hear. However, it must be remembered that the WSB argument can cut both ways. As indicated in the general equilibrium analysis, if there is a WSB in other types of work and it is ignored, the optimal amount of labor for the fishing industry can be overestimated. In analyzing a WSB, the well-known fact that an optimal allocation of resources in a commercial fishery will be achieved if the net economic or social surplus from its operation is maximized has been reiterated. More important it has been stressed that it is crucial to specify properly what constitutes social surplus. Four component types of surplus were discussed. They were industry profits, which at MEY is the rent due to the unique capital in the fish stock, normal factor rent, consumer surplus, and worker satisfaction bonus. Regulation to reduce the amount of fishing activity will always lead to decreased rents to the fishery labor force, and may well decrease the consumer surplus to fish consumers as well, even though there will be a net increase in the value of goods and services produced.

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Growth Rates of a New Cell Line from Channel Catfish Ovary and Channel Catfish Virus Replication at Different Temperatures

P. R. BOWSER[1] AND J. A. PLUMB

Department of Fisheries and Allied Aquacultures, Agricultural Experiment Station, Auburn University, Auburn, AL 36830, USA

BOWSER, P. R., AND J. A. PLUMB. 1980. Growth rates of a new cell line from channel catfish ovary and channel catfish virus replication at different temperatures. Can. J. Fish. Aquat. Sci. 37: 871–873.

Growth rates of a new cell line from the channel catfish, *Ictalurus punctatus*, were determined at temperatures from 10 to 35°C. Growth was more rapid at 35°C with the cells having a population doubling time of 12 h, and cell numbers declined at 10°C. The cells survived frozen storage at −70°C for up to 6 mo. Replication of channel catfish virus occurred from 10 to 35°C. Although it was most rapid at 35°C, maximum replication occurred at 30°C.

Key words: fish viruses, channel catfish virus, herpesvirus, fish cell cultures, fish diseases, cell growth rates

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Les taux de croissance d'une nouvelle lignée de cellules de barbue de rivière, *Ictalurus punctatus*, furent déterminés à des températures allant de 10 à 35°C. La croissance est plus rapide à 35°C, les cellules ayant un temps de doublement de population de 12 h, alors qu'à 10°C, le nombre des cellules diminue. Les cellules survivent à un entreposage congelé à −70°C sur une période allant jusqu'à 6 mois. La reproduction du virus de la barbue de rivière se produit entre 10 et 35°C. Bien qu'elle se produise avec le plus de rapidité à 35°C, c'est à 30°C que la reproduction est maximale.

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NUMEROUS new cell lines have been developed recently to facilitate research in the area of viral diseases of fish (Wolf and McAllister 1978). One such new fish cell line is the channel catfish ovary (CCO) line developed from the ovaries of a healthy juvenile channel catfish, *Ictalurus punctatus* (Bowser 1978). This cell line was initiated to provide a cell line from the host species with which channel catfish virus (CCV) could be studied. The purpose of this study was to provide information for the routine handling of CCO cells and to describe the ability of CCO cells to support the replication of CCV.

Materials and methods — CCO cells (passages 109 and 150) were grown in Leighton tube coverslip cultures to determine cell growth rates. The culture medium was Eagle's minimal essential medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (pencillin, 100 IU/mL; streptomycin, 100 μg/mL, gentamicin, 50 μg/mL) (MEM-10). Tubes were seeded with CCO cells in 1.0 mL of MEM-10 at the density of 100 cells/mm² of coverslip surface. Cultures were incubated at 25°C for 1 h to allow cell attachment. Following this initial incubation, two cultures were fixed in 10% formalin and stained for counting. Wet mounts were made of the inverted coverslip over the grid area of a hemacytometer and five, 1-mm² areas were counted on each coverslip. This gave the initial seeding density.

The remaining cultures were divided into groups and incubated at 10, 20, 30, and 35°C at pH 7.2–7.4. At intervals of 1 wk at 10°C, 2 d at 20°C, and 12 h at 30 and 35°C, duplicate cultures were fixed, stained, and counted, using 2.5% trypan blue to determine cell viability by the method of Greene et al. (1964).

Plating efficiency was determined by making 10-fold dilutions of CCO cell suspensions (passage 113) in MEM-10 and determining the number of viable cells (Greene et al. 1964). A microculture plate was seeded with 0.2 mL per well of the dilutions containing 390 and 39 viable cells per inoculation. Sixteen replicate wells were inoculated for each dilution. The cultures were incubated at 30°C for 24 h with a pH of 7.2–7.4. Following incubation, the number of colonies of CCO cells were counted for each culture well and the absolute plating efficiency (Federoff 1966) was determined.

CCO cells were tested for viability following freezing. Cells (passage 76) were digested from culture flasks with

[1]Present address: University of California, Bodega Marine Laboratory, Bodega Bay, CA 94923, USA.

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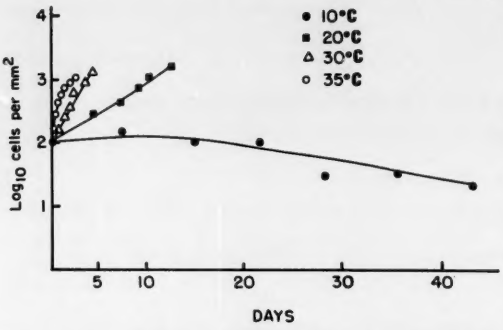

FIG. 1. Growth rates of channel catfish ovary cells at 10, 20, 30, and 35°C in Eagle's minimum essential medium–10% fetal bovine serum, at pH 7.2–7.4.

0.1% trypsin and 1:5000 versene solution and resuspended in a freezing medium consisting of 90% FBS and 10% dimethylsulfoxide (DMSO). The cell suspension was sealed in 2-mL ampules. Ampules of cells were held at 5°C for 1 h, then reduced to −20°C for 1 h, followed by transferring to −70°C for storage. At monthly intervals for 6 mo, ampules were quickly thawed and the cells seeded into 25-cm² culture flasks with 5 mL of MEM-10, and were observed for viability.

In a later evaluation, CCO cells in passage 115 were prepared for freezing as above. Prior to freezing, viability counts were made of the cells using a hemacytometer and the trypan blue exclusion technique (Greene et al. 1964). At 1, 7, and 30 d, the cells were thawed, centrifuged at 150 g for 5 min at 25°C, and resuspended in an equal amount of MEM-10. Viability determinations were made as before.

CCO cells from passages 100–106 and 150 were infected with CCV, and cell-associated and released virus were titrated at regular intervals. Medium was aspirated from CCO monolayers in 60-mm culture dishes and the cells were infected with 0.5 mL of CCV suspension with $10^{6.3}$ tissue culture infectious doses 50% end point ($TCID_{50}$), providing a multiplicity of 0.5 virions per cell, and allowed to adsorb for 1 h at 25°C. Following adsorption, the cultures were washed three times with 5 mL of Hanks' balanced salts solution (HBSS) and then 5 mL of MEM-10 was added to each dish. The cultures were incubated at 10, 20, and 30° and later at 35°C, at pH 7.2–7.4.

Results — CCO cells grew most rapidly at 30 and 35°C (Fig. 1). The cells grew well, but more slowly at 20°C, while at 10°C the number of cells declined slightly. CCO cells did not attach or survive at 40°C. The population doubling times of the cell cultures were 12 h at 35°C, 15.2 h at 30°C, and 2.6 d at 20°C. Viability of the cells ranged from 94 to 98% for all temperatures.

The absolute plating efficiency of the CCO cells was 23%. This number indicates the percentage of viable cells which gave rise to colonies of cells when seeded into culture in MEM-10 at 30°C. With time, the colonies expanded to form monolayer cultures. Many other cells attached but did not divide.

Frozen CCO cell cultures (passage 76) successfully recovered 6 mo after storage at −70°C. Longer storage was not tested. After storage at −70°C for 1 d, 75% of the CCO cells (passage 115) were still viable. After 7 and 30 d of frozen storage, the recovery of viable cells was 58 and 36%, respectively.

The one-step growth-curve studies of CCV in CCO cell cultures showed that released and cell-associated virus were not significantly different when data were plotted on probability paper (Cassie 1954). As expected, the cell-associated virus titers were higher than cell-free virus during the early sampling periods, but during peak titers they were similar. This was similar to that of Wolf and Darlington (1971) for CCV growth in Brown Bullhead (BB) (*I. nebulosus*) cells. At 35°C, virus production was very rapid reaching a peak titer of $10^{6.83}$ $TCID_{50}$/mL at 10 h postinfection (PI) (Fig. 2). Greatest virus release occurred between 4 and 6 h PI which corresponds to total cytopathic effect (CPE) of the cell sheets. Virus production was slightly slower at 30°C where virus release peaked at 16 h, but the titer was over 1 log greater than at 35°C. At 20°C, virus decreased during the first 12 h, then entered the logarithmic phase until a peak titer of $10^{5.5}$ $TCID_{50}$/mL was reached ~48 h PI. At 35, 30, and 20°C, the end of the log phase coincided with maximal CPE, char-

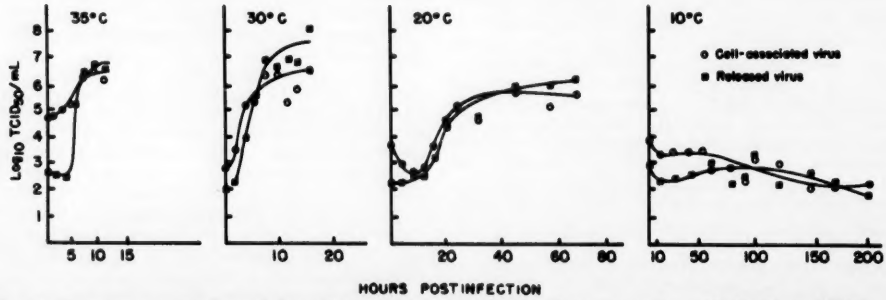

FIG. 2. One-step growth curve of channel catfish virus in channel catfish ovary cells at 10, 20, 30, and 35°C.

acterized by the formation of syncytia. At 10°C, virus production was very low, reaching a peak titer of $10^{3.4}$ $TCID_{50}$/mL at ~48 h postinfection. After 48 h, viral infectivity declined steadily.

Discussion — This study provides some guidelines for the handling of CCO cells in the laboratory and their use in CCV diagnosis and research. The cells could be cultured over a temperature range of 10 to 35°C, with the cells growing most rapidly at 35°C, but to a greater density at 30°C. A monolayer occurred when there was ~10^3 cells/mm^2. CCO cells can be held at −70°C for extended storage using an FBS–DMSO freezing medium.

CCV replication curves in CCO cells showed virus production to be most rapid at 35°C, but again virus titers were greatest at 30°C, which is near the optimum temperature for CCV disease epizootics (Plumb 1977). At 35°C initial focal CPE was visually detectable at 2 h PI and there was total cell culture involvement at 4 h PI. With decreasing temperature, the production of CCV in CCO cells decreased. Cell-associated and released virus were not different through the growth curves at each temperature. In view of the 35°C tolerance of CCO cells and their capability of replicating CCV at that temperature so rapidly, it is suggested that for CCV diagnostic purposes, attempts at isolating the virus from disease cases should include incubation of inoculated cultures at 35°C. If one desires to produce a stock of CCV with a high titer, incubation should be at 30°C and the virus harvested when the cell cultures show a maximum involvement in syncytia formation. Following this point, infective viral particles decline in number.

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Effects of Unconventional Size Limits on the Growth Rate of Trout

L. D. Favro and P. K. Kuo

Department of Physics, Wayne State University, Detroit, MI 48202, USA

and J. F. McDonald

Department of Mathematics, University of Windsor, Windsor, Ont. N9B 3P4

Favro, L. D., P. K. Kuo, and J. F. McDonald. 1980. Effects of unconventional size limits on the growth rate of trout. Can. J. Fish. Aquat. Sci. 37: 873–876.

A computer model was used to study the genetic effects on a trout population that was subjected to selective fishing with unconventional size limits; both a minimum and maximum size were specified. The model predicts that such window size limits will not have their intended effect, i.e. to increase the percentage of larger fish in the population.

Key words: fish, trout, genetics, growth, size limit, window size limit, *Salmo trutta*

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Nous nous sommes servi d'un modèle d'ordinateur pour étudier les effets génétiques, sur une population de truites, d'une pêche sélective avec tailles limites non conventionnelles, les tailles minimale aussi bien que maximale étant spécifiées. Le modèle prédit qu'une telle mesure n'aura pas l'effet anticipé, i.e. un plus fort pourcentage de grands poissons dans la population.

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POPULATIONS of wild trout subjected to intense recreational fishing pressure often show a systematic decrease in the percentage of larger fish. For example, see the data presented in Favro et al. (1979). It has frequently been suggested that these effects might be reversed by instituting unconventional size limits in the fishing regulations. These suggestions usually involve either an "inverse" limit, which specifies a maximum rather than a minimum size for the fisherman to keep his catch, or a "window" limit, in which only fish between some minimum and maximum sizes may be kept. In fact, such unconventional regulations have been put into effect in some areas. For example, an inverse size limit with a maximum size of 14 in. (1 in. = 25.4 mm) has been in effect for several years on a stretch of Henry's Fork of the Snake River in Idaho. Also, a window size limit from 8 to 12 in. was instituted this year on a stretch of the Au Sable River in Michigan.

Our previous work (Favro et al. 1979) has shown that the changes which take place in the size distribution of a wild trout population under conventional regulations are consistent with the predictions of a computer model which attributes these changes to genetic effects. In view of the time and expense involved in assessing the impact of unconventional size limits by field testing, it would seem prudent to use a similar model to attempt to foresee the effects of these limits. This note is to report the predictions of such a computer model.

The model assumes that the growth rate of trout is an inherited trait primarily controlled by a few genes. It assumes that the legal size range is a window with adjustable upper and lower limits. With the lower limit set sufficiently small, the window is equivalent to an inverse limit. With this type of size limit, several new variations of the fishing-induced genetic selection can occur. For instance, it may be that some fast growing fish pass completely through the window at a time of year when fishing is not allowed and thus escape fishing mortality completely. Others may spend only a short time in this window. To accommodate these possibilities we have changed the computer model so that growth and mortality are calculated weekly rather than yearly and have modified our growth curves to account for seasonal variations in growth rates. The calculations presented here use a sinusoidal variation of growth rate over the year with a 3:1 ratio of peak summer growth to minimum winter growth in agreement with preliminary data taken on the Au Sable River. The yearly average growth curve is the same as the one used in the previous paper. A 5-mo (May through September) fishing season is assumed. Also, with a window limit the degree of dependence of fishing mortality on the growth rate becomes an important factor. This is because fish that spend only a short time in the legal size window may still be selectively killed if their food intake is great enough to make them particularly susceptible to angling. To evaluate these various possibilities we have modified the original program to calculate the expected population size distributions both with and without an upper size limit and both with and without a growth-rate dependence in the fishing

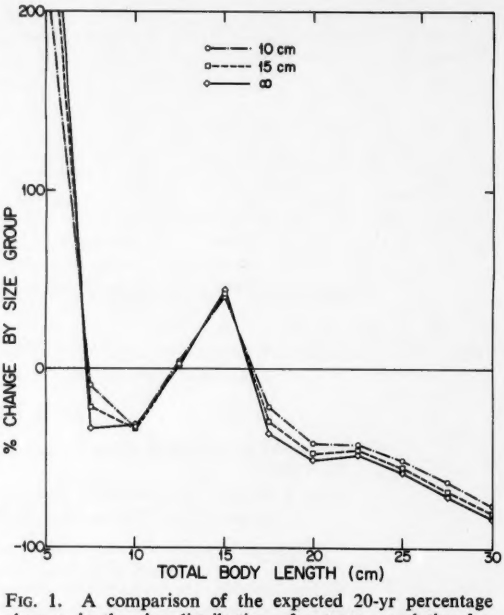

FIG. 1. A comparison of the expected 20-yr percentage changes in the size distribution of a trout population between conventional and window size limits. The lower limit is taken as 20 cm. The width of the window is taken to be 10 cm, 15 cm, and ∞ (i.e. conventional size limit). The fishing mortality is growth-rate dependent.

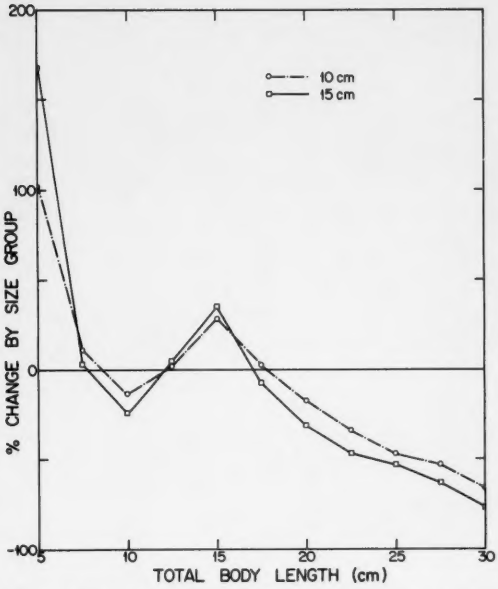

FIG. 2. The expected 20-yr percentage changes in the size distribution of a trout population for the window size limits where the lower limit is 20 cm and the width of the window is 10 cm and 15 cm. The fishing mortality is independent of growth rate.

mortality rate. Because of the great variety of possible combinations of the various parameters involved, no attempt has been made to present an exhaustive compilation of the results here. Instead, in the next section we give a selection of typical results intended to indicate the general character of the response of a trout population to changes in these parameters.

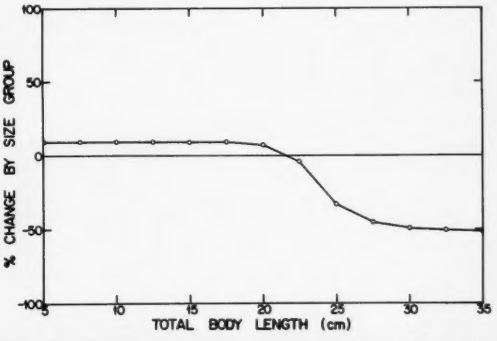

FIG. 3. The expected 20-yr percentage change in the size distribution of a trout population with a 20- to 30-cm window size limit with the genetic effects of selective fishing removed from the model.

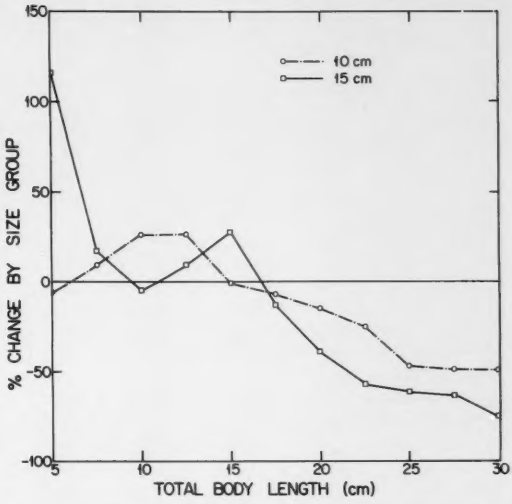

FIG. 4. The expected 20-yr percentage changes in the size distribution of a trout population for the window size limits where the lower limit is 15 cm and the width of the window is 10 cm and 15 cm. The fishing mortality is independent of growth rate.

Results — Results of calculations with our modified model are presented as graphs of percentage change in the population from the initial population. Figure 1 provides a comparison between a conventional size limit (no upper limit) and a window size limit. The lower limit, below which fish may not be killed, is taken as 20 cm in all cases. The upper limit is taken to be 30 cm, 35 cm, and ∞ (no upper limit). The curves are all calculated for a 20-yr time span, and the fishing mortality is taken to be 40% (as defined in the previous paper). There is remarkably little difference between the final size distributions calculated with the various upper size limits indicating a lack of sensitivity to this parameter.

Figure 2 shows similar curves but with the growth-rate dependence of the fishing mortality removed. Although there are quantitative differences between these curves and the growth-rate-dependent curves of Fig. 1, their overall behavior is quite similar. In fact, one can compensate for the changes that result from varying the growth rate dependence by varying the fishing parameter.

Figure 3, on the other hand, shows the result of calculations with the same model but with the genetic content removed. That is, Fig. 3 was calculated assuming that the growth rate was strictly nonheritable. It shows a final population change which is uniformly above the initial population for sizes up to the minimum size limit, which steadily decreases for sizes between the two limits and which levels out again for sizes above

the maximum limit. This behavior is in striking contrast to the oscillatory behavior predicted by the genetic model and observed in the data.

Finally, Fig. 4 displays the calculated population changes which occur with a very small (15 cm) minimum size limit and two different window sizes (10 and 15 cm). Both are calculated with a growth-rate-independent fishing mortality. The other parameters are the same as those in Fig. 2. These two curves differ considerably: the one with the larger (15 cm) window resembling the previous genetic calculations, Fig. 1 and Fig. 2, and the one with the smaller (10 cm) window bearing some resemblance to the curve of Fig. 3 in which the genetic content has been removed. This resemblance to Fig. 3 results from the fact that this particular choice of parameters is close to the transition point where selection *against* faster growing fish changes to selection *in favor* of faster growing fish because they pass through the window faster. In fact, the dip in the curve at very small sizes (less than 10 cm) is an indication of a small amount of selection against slower growing fish. It should be emphasized, however, that this result was obtained only by making both the lower limit and the window size very small and by making the unrealistic assumption that slow growing fish are as equally likely to be caught as fast growing fish of the same size. We have found no reasonable combination of parameters which exhibits a selection in favor of fast growing fish when a growth-rate-dependent fishing mortality is used.

Discussion — Our calculations have shown that with reasonable choices of numbers, window size limits can be expected to produce the same depression in the percentage of larger fish as that produced by conventional limits. The introduction of these unconventional size limits will of course produce changes in the details of the population structure. However, it is not expected that these experimental size-limit regulations can, by themselves, reverse the genetic changes which may have taken place in the population as a result of previous selective fishing.

FAVRO, L. D., P. K. KUO, AND J. F. MCDONALD. 1979. Population–genetic study of the effects of selective fishing on the growth rate of trout. J. Fish. Res. Board Can. 36: 552–561.

PERSPECTIVES

Temporal and Spatial Scales in Phytoplankton Ecology.[1] Mechanisms, Methods, Models, and Management

GRAHAM P. HARRIS

Department of Biology, McMaster University, Hamilton, Ont.

HARRIS, G. P. 1980. Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models, and management. Can. J. Fish. Aquat. Sci. 37: 877–900.

This article is essentially a review of the temporal and spatial scales of variability in both marine and freshwater planktonic environments and the algal responses to those scales. I assert that there are problems with our present understanding of these scales and the use of inappropriate assumptions concerning the occurrence of steady-state conditions. In a nonsteady-state environment the concepts of limiting nutrients must be changed, and the extrapolation from culture to field conditions is fraught with problems. In this paper I review the evidence for the existence and importance of small-scale, high frequency and large-scale, low frequency variation in the planktonic environment and show that such variation fundamentally affects our understanding of existing processes. Methodology and models must also reflect the true scales of variability which exist. I show that there are, at present, problems with our understanding of planktonic processes which greatly affect our ability to manage water quality. New concepts and models are urgently needed. Finally I propose a new model of community structure and process in variable environments which accounts for the correct 'algal' scales of perturbation and response and allows certain predictions to be made. It is possible to reconcile certain problems and controversies in the literature by the use of such a model. An enhanced ability to manage planktonic systems should result from an improved understanding of the true scales of variability which exist.

Key words: lakes, oceans, phytoplankton, communities, nutrients, models, management, eutrophication, fluctuations, scales

HARRIS, G. P. 1980. Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models, and management. Can. J. Fish. Aquat. Sci. 37: 877–900.

Le présent article est essentiellement une revue des échelles de variabilité temporelle et spatiale dans des environnements tant marins que d'eau douce et des réponses algales à ces échelles. Je soutiens que notre compréhension actuelle de ces échelles et l'utilisation d'hypothèses inappropriées concernant l'occurrence de conditions d'équilibre laissent à désirer. Dans un environnement non en équilibre, il faudra modifier les notions d'éléments nutritifs limitatifs, et l'extrapolation de conditions d'élevage à des conditions sur le terrain est pleine de danger. Je passe ici en revue les preuves de l'existence et de l'importance d'une petite échelle, d'une haute fréquence et grande échelle et enfin d'une variation à faible fréquence de l'environnement planctonique; je démontre de plus qu'une telle variation affecte fondamentalement notre compréhension des processus actuels. La méthodologie et les modèles doivent aussi refléter les échelles de variabilité qui existent dans la réalité. Je démontre l'existence de problèmes touchant notre compréhension des processus planctoniques, influant grandement sur notre aptitude à gérer la qualité de l'eau. Il existe un besoin urgent de concepts et de modèles nouveaux. Finalement, je propose un nouveau modèle de structure et de processus communautaires dans des environnements variables, modèle qui tient compte d'échelles «algales» correctes de perturbation et de réponses, et qui permet certains prédictions. L'utilisation d'un modèle de ce genre permet de réconcilier certains problèmes et controverses exposés dans des travaux

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publiés. Une meilleure compréhension des échelles réelles de variabilité devrait améliorer notre aptitude à gérer les systèmes planctoniques.

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THIS review is an attempt to bring together some thoughts on how the existence of a number of spatial and temporal scales of variability in planktonic environments may affect the ecology of the phytoplankton. In a turbulent environment horizontal and vertical diffusion ensure that space and time are interrelated (Bowden 1970; Boyce 1974; Okubo 1971), so the discussion of spatial and temporal scales cannot be separated.

The understanding of role of environmental variability in planktonic environments is, I believe, central to an understanding of how planktonic systems behave. One way to examine the variability in space and in time which exists in complex ecological systems is to think of the variability in terms of hierarchy of processes operating at different temporal and spatial scales.

In the sense of hierarchy theory, complex (eco) systems are usually classified and decomposed by level and by frequency (Simon 1973). At the lowest levels processes are operating at high frequencies so the lowest level subsystems appear to be at equilibrium over the time span of interest. In the middle range of level and frequency the observable dynamics are not assumed to be at steady-state and must therefore be studied and characterized. At the largest scales (low frequency) the period may be so long that the properties of the system may appear to be constant over the period of study. What I am really asking is that we change our ideas about what is at equilibrium, what the observable dynamics are, and what may be regarded as constant. The range of observable dynamics must be extended both upwards and downwards in the hierarchy.

Over the years our concepts of the observable dynamics of planktonic ecosystems have changed but an identifiable paradigm has evolved. The paradigm which emerges (Kuhn 1970) determines how we view the world, how experiments are performed, and how we interpret the results. In this review I want to do a number of things. Firstly, I want to review some of the basic assumptions which have become the paradigm for our research into the ecology of phytoplankton over the last 20 yr or so. Secondly, I want to discuss some new methods and ideas which may well result in the modification of that paradigm. Thirdly, I want to review some of our modeling and management strategies which are based on the accepted paradigm and which may now need revision. Lastly, I will offer some personal views and opinions as to the way in which our understanding may change in the future.

I have used both limnological and oceanographic information to develop the arguments presented below as I seek general rules for the operation of planktonic communities. I do not expect everyone to agree with all the views presented herein. I have attempted to present the argument as concisely as possible, and the article is written as a contribution to the debate and as a spur to further research.

The Old Paradigm

SCALES

The earliest views of the planktonic environment were that of an isotropic homogeneous environment at equilibrium over large scales. These views were, however, questioned some years ago by Hutchinson (1961), who wondered how so many species coexisted in such environments. Later Richerson et al. (1970) provided an explanation in the form of a hypothesis postulating "contemporaneous disequilibrium." This hypothesis assumed that the planktonic environment was not homogeneous and that the temporal and spatial scales of variability in the water disrupted any approach to competitive exclusion (Grenney et al. 1973) (i.e. the range of observable dynamics was extended to smaller scales). The observation of patchiness in planktonic communities is not new (cf. Hardy 1935, 1936) but great impetus was given to the work by the development of in vivo fluorescence (IVF) techniques for the measurement of chlorophyll (Lorenzen 1966; Herman and Denman 1977; Denman and Mackas 1978). Pioneering work on spatial scales by Platt and others (Platt 1972; Platt et al. 1970) and the introduction of spectral analysis techniques (Platt and Denman 1975a; Fasham 1978) has led to the publication of many papers on the spatial distributions of phytoplankton biomass in lakes and oceans (Denman 1976, 1977; Denman and Platt 1974, 1976; Denman et al. 1977; Estrada and Wagensburg 1977; Fasham and Pugh 1976; Horwood 1978; Lekan and Wilson 1978; Platt 1975, 1978; Platt and Denman 1975b; Powell et al. 1975; Richerson et al. 1975, 1978).

But which scales are important? Observations of patchiness using IVF techniques gave power spectra in which the greatest variability occurred at the largest scales. In lakes the largest scales appeared to be associated with basin dimensions as would be expected (Boyce 1974; Richerson et al. 1978). In the oceans and lakes theoretical explanation of the spectra was provided by the "KISS" model of Kierstead, Slobodkin, and Skellam (Kierstead and Slobodkin 1953; Skellam 1951). The KISS model relates the rate of horizontal diffusion to the presumed growth rate of the algae and calculates the critical patch size below which diffusion destroys any growth effects. To do this certain assumptions were made. These involved the use of IVF as an indicator of algal biomass, the description of algal ecology in terms of total biomass rather than individual species, and the use of a model with a single growth rate value for all the biomass. Given these assumptions

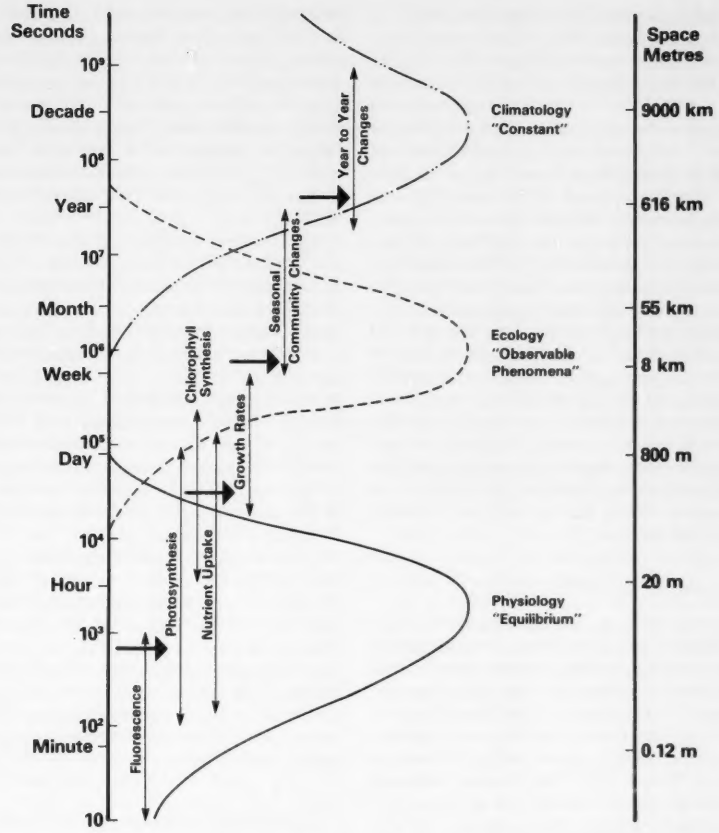

FIG. 1. A summary of the hierarchy of the various algal responses to the spectrum of environmental fluctuations. The temporal and spatial scales are linked by the processes of horizontal turbulent diffusion (Bowden 1970). The three bell curves roughly define the scales of interest to physiologists, ecologists, and climatologists. The horizontal arrows are meant to show that higher frequency (lower level) processes collapse into higher level responses. The figure does not pretend to be an exhaustive description — for more details and references see text.

(for the time being) the theory and the observations appeared to be in agreement. According to the KISS model any patch of algae <100 m in size will be destroyed rapidly by horizontal diffusion (Okubo 1978) as the algae cannot grow sufficiently rapidly to overcome the rates of horizontal diffusion which exist in the environment (Denman and Platt 1976; Platt 1975; Platt and Denman 1975b). Grazing was added to the model but it did little to modify the basic argument. The theory predicted that at scales >1 km algal growth could overcome the effects of turbulent diffusion and some of the field data appeared to show this effect. This bend in the slope of the IVF spectra at scales $\simeq 1$ km has become known as "Platt's knee."

The link between space and time which the KISS model provides is the link between the growth rate of the algae and the rate of horizontal diffusion. Dye diffusion studies give the relationship between patch size, time, and diffusion rate so that 1 km is equivalent to a time scale of about 1 d (Bowden 1970; Boyce 1974; Okubo 1971) (Fig. 1). One day was accepted as a reasonable estimate for the maximum division rate of planktonic algae (Kierstead and Slobodkin 1953; Skellam 1951).

A number of model formulations seemed to converge on this one solution: that at scales of about 1 km

or greater biological processes outweigh the effects of horizontal diffusion (Dubois 1975a, b; Levin and Segel 1976; Okubo 1974, 1978; Steele 1974a, b; Wroblewski and O'Brien 1976; Wroblewski et al. 1975; Ford and Thornton 1979). In this way temporal and spatial scales of a day or so and about 1 km have been accepted as the most important small horizontal scales in limnology and oceanography. There have been reports of data which show no "knee" (Horwood 1978) but the spectra which have been produced certainly show that large-scale, slow processes dominate the variance. It has therefore been assumed that smaller, higher frequency effects are relatively unimportant and that growth-related events determine the spatial structure of the communities (Platt and Denman 1975a). That the role of smaller, higher frequency processes is poorly understood (Steele 1974b) and largely neglected is evident from the proceedings of a recent NATO school (Steele 1978). The reports of substantial and significant high frequency variability in the biomass of planktonic systems appear to come from observations made in mixing zones (Haffner et al. 1979; Harris et al. 1979a, b, c; Harris and Piccinin 1979; Harris and Smith 1977; McAlice 1970; Zarull 1979).

Growth Rates and Physiology

The KISS model, with its assumptions about rates of horizontal diffusion and growth rates of the phytoplankton (Okubo 1978), forms the theoretical basis for work on biomass distributions. But what are the in situ growth rates of the plankton? Much of the data for the influence of environmental factors on phytoplankton comes from growth experiments in batch or continuous culture. Rhee (1979) has recently reviewed the use of continuous culture techniques on algae and Eppley (1972) and Goldman and Carpenter (1974) have reviewed the data on temperature effects on growth. There is, however, little field data available (Goldman et al. 1979). Much of the growth rate data, of late, has come from continuous culture techniques because of the improved environmental control of steady-state (reviewed by Rhee 1979). The implicit assumption is that equilibrium exists at temporal scales less than the doubling time of the population (cf. Ford and Thornton 1979). The use of steady-state continuous culture techniques has, in itself, also tended to determine the interpretation of the data because of the widespread use of Michaelis–Menten type kinetics to describe the data. It should be remembered that such kinetics only are a way to *describe* the data, they do not imply a causal mechanism, as was pointed by MacIssac and Dugdale (1969). The continuous culture data (and also to some extent the batch culture data) have allowed us to arrive at data defining the maximum growth rate (μ_{max}) and the half-saturation constant for growth (K_s) limited by the major nutrients (Rhee 1979). From such results it became clear that the apparent concentrations of nutrients in lakes and oceans were often below K_s and would therefore imply frequent P limitation in freshwater and frequent N limitation in the oceans. From the continuous culture data it also became possible, apparently, to extrapolate to the field situation and to make some statements about possible in situ growth rates. These in situ growth rates were frequently assumed to be below the maximum growth rates (μ_{max}) which could be achieved in the absence of nutrient limitation (for example, cf. Eppley et al. 1973).

The modern concepts of oligotrophy and eutrophy also to some extent rest on assumptions such as these. The concept of single limiting nutrient having an overall large-scale effect on algal growth rates and biomass (yield) arose from the use of large-scale growth-related conceptual models (cf. preamble to Ryther and Dunstan 1973). Lakes have often been looked upon as large batch or continuous culture systems in which nutrient inputs are converted to a total algal yield in surface waters in summer (Vollenweider 1968, 1975, 1976). The central ocean gyres have also been thought of as large continuous culture systems (Eppley et al. 1973) and may come close to being so (Goldman et al. 1979) on a long-term basis.

Thus a quasi steady-state view of phytoplankton ecology has led to a paradigm in which the role of variations in the environment at periods less than 1 d or longer than a year or so has been emphasized less than the mid-range of variations (Fig. 1). This, coupled with a sampling routine which resolves only such scales, immediately biases the whole emphasis of phytoplankton ecology. The relationship between sampling strategies and interpretation was eloquently discussed by Stommel (1963).

Extending the Range of Observable Dynamics

Long-Term Changes in Phytoplankton Population

I will begin with a discussion of large-scale, long-term processes and then turn to smaller and smaller scales (cf. Fig. 1).

Over the last few years meteorologists have begun to understand the larger-scale atmospheric processes which determine global weather patterns (Lamb 1972, 1977), and a recent series of papers has discussed effects of such processes on oceanic life (Cushing 1978; Cushing and Dickson 1976; Garrod and Colebrook 1978; Smith 1978). There are very few long-term data sets in existence in the literature resulting from the direct observation of planktonic communities. The best sets of published data are undoubtably fisheries data; some records go back as far as the sixteenth century (Cushing 1978; Cushing and Dickson 1976; Smith 1978). For phytoplankton communities the longest continuous records appear to be those of Lund from the English Lakes (Lund 1954, 1955, 1964, 1966, 1969, 1971, 1978; Macan 1970; Mortimer 1969).

Palaeolimnological methods have concentrated on

diatom communities and there are many examples of postglacial changes in such communities. The body of literature on such topics is beyond the scope of this review partly because of the problem of deciding which species are truly planktonic and which benthic. It is my impression that the best documented series of postglacial sedimentary records are those from the English Lake district and associated areas of North-west England, Wales, Scotland, and Ireland (Battarbee 1973, 1977; Elner and Happey-Wood 1978; Haworth 1969, 1972, 1976; O'Sullivan et al. 1973; Pennington 1970, 1973; Round 1957, 1961; Smith 1970; Turner 1970). These records are particularly complete because of the wealth of climatological and historical data which together make the changes in the diatom communities of the lakes understandable (Lamb 1977; Pennington 1970; Smith 1970; Turner 1970).

One important feature of long-term records discussed by Cushing (1978) and Cushing and Dickson (1976) is the rectification of long-term climate trends by planktonic ecosystems, i.e. in the presence of long-term, gradual changes in climate, the planktonic communities show a "flip-flop" effect. The best example of this appears to be the so-called Russell cycle (Russell 1973; Cushing and Dickson 1976; Russell et al. 1971) in which the planktonic ecosystem of the English Channel changed dramatically in the period from 1925 to 1935 and then flipped back again to the pre-1925 condition in the period from 1965 to 1975. The overall climate change during this period was small. The N.E. Atlantic warmed (and subsequently cooled) by about 0.5°C during this period (Cushing 1978). There are other reports of similar phenomena in planktonic communities; the switch between communities dominated by *Ceratium* and those dominated by *Microcystis* in an English mere (Livingstone and Cambray 1978; Reynolds 1978a) may represent a similar phenomenon. Long-term changes in nutrient loading may produce similar "flip-flop" effects.

Cushing (1978) and Cushing and Dickson (1976) explain such "flip-flop" effects by means of their "match–mismatch" hypothesis in which the phasing of seasonal activities is stressed. When two events are in phase the result may be quite different from that when the two events are partially or completely out of phase. For events which occur over relatively short periods at one time in the year (i.e. the spring phytoplankton bloom and spawning, Cushing 1978; Cushing and Dickson 1976), correct phasing is essential.

The "match–mismatch" hypothesis may be extended to explain a number of events in fluctuating environments, so the full understanding of fluctuating environmental conditions requires a knowledge of the mean level, amplitude, period, and phase. I will expand on this in the final section. I merely wish to state here that long-term, large-scale events cannot necessarily be treated as constant, as the phasing of lower level (higher frequency) events in the hierarchy may cause dramatic changes at higher levels. Also our expanding knowledge of climatology is leading us to treat the global climate as a system which varies at a number of scales (Lamb 1972, 1977; Newell 1979) — scales which interact with the planktonic ecosystems of lakes and oceans in complex and poorly understood ways (Cushing 1978; Cushing and Dickson 1976; Garrod and Colebrook 1978; Smith 1978).

Changes in the nutrient supply to lakes over postglacial time periods result in changes in the diatom communities preserved in the sediments (cf. for example, Battarbee 1973, 1977; Haworth 1969; Round 1961; Stoermer 1977). These changes may also show the rectified changes that some of the climatically induced cycles show. Such changes may be related to the fact that the rapid nutrient recycle mechanisms within the lakes ensure that the community and biomass changes are a high level averaged response and that they are at equilibrium with the nutrient supply. Peters (1979) has shown a similar effect recently down a nutrient gradient in one lake. This would also tend to indicate that community structures evolve in such a way as to utilize efficiently the available nutrient supply (see below).

The spatial and temporal distribution of a nutrient such as nitrogen or phosphorus will be controlled by a number of intrinsic physical factors peculiar to each body of water. The mean value may change over time, but the period, amplitude, and phase may not. Clearly phytoplankton communities are sensitive to more than the mean value of any given parameter so proportional changes to a changed loading may be observed as a change in biomass, but the species composition may be relatively insensitive to such changes. Also nutrients are not the only factors which control phytoplankton growth.

Community Structure and Biomass Distributors

These are problems with the interpretation of some aspects of the data concerning the spatial variability of communities. The data are based on in vivo fluorescence techniques and the problem lies in the assumptions mentioned above. Zarull (1979) showed that IVF techniques tend to underestimate the importance of the high frequency variability in the real system and, as might be expected, the spatial distribution of IVF is not the same as that of total chlorophyll. IVF per unit chlorophyll varies over a number of time scales in nature (Blasco 1973; Harris 1979a; Heaney 1978; Kiefer 1973a, b; Loftus and Seliger 1975). Also, as the cellular content of chlorophyll varies between individuals and between species, Zarull (1979) found that the distribution of the individual species did not match the spatial distribution of the total chlorophyll. Sandusky and Horne (1978) also concluded that when several species are present the pattern in the biomass underestimates the true degree of spatial organization in the phytoplankton.

Although the role of small-scale, high frequency

events may be underestimated by IVF techniques, the spectra so produced do certainly show that the greatest patchiness occurs at the largest scales. Biomass distributions average out the individual species distributions so that fluctuations appear at longer time scales and larger spatial scales. Clearly the fluctuations in the physical and chemical environment reduce the competitive interactions between species and so allow more species to coexist (Grenny et al. 1973; Hulburt and Horton 1973; Richerson et al. 1970). This is not a property which is unique to planktonic systems (Levin 1976), so we are faced with the identification of the correct temporal and spatial scales by which to explain the community structure. The work on biomass provides some clue as to the correct scales, but we must look further.

Nutrient Uptake and Growth

The interpretation of the growth rate data is no easier. Problems arise when explicitly steady-state continuous culture techniques are compared with the natural nonsteady-state situation. This point has been made before by Jannasch (1974) and again by Rhee (1979). Continuous culture techniques assume (largely for mathematical simplicity) that at steady-state only one nutrient is limiting, that the vessel is completely mixed, and that a constant proportion of the cells are viable (cf. the excellent review of Rhee 1979). Furthermore, Michaelis–Menten kinetics assume constant stoichiometry and yield for the single species in question. Clearly none of these assumptions hold for mixed natural populations (Jannasch 1974) and the different species in the natural assemblage will not necessarily have identical kinetic parameters (Williams 1973).

A number of years ago data began to appear which Michaelis–Menten kinetics did not explain (Toetz et al. 1973), in particular, data which showed that growth rates were dependent on both internal and external nutrient concentrations (reviewed in Rhee 1979 but cf. Droop 1968, 1973, 1974, 1975; Goldman 1977a; Rhee 1972, 1973; Tilman and Kilham 1976). For instance, growth may continue for a while in the absence of an external nutrient supply (Mackereth 1953; Rhee 1972) or growth may not begin until uptake has taken place (Lean and Nalewajko 1976). This problem was circumvented by the formulation of Droop (1968, 1973, 1974, 1975), which used a relationship between growth rate and the cell quota of the limiting nutrient.

At steady-state the Michaelis–Menten and Droop formulations, however, are equivalent (Burmaster 1979a, b; DiToro 1979; Goldman 1977a; Goldman and McCarthy 1978; Rhee 1979). Phytoplankton have a high affinity for nutrients and the rate of uptake may, for short periods (less than the generation time), far exceed the long-term growth requirements of the cell. The true K_s of a population is only obtained when cells are completely starved of that particular limiting nutrient (Rhee 1979). K_s (the half-saturation constant for growth) is usually much smaller than K_m, the affinity constant for uptake (reviewed in DiToro 1979). There is evidence that K_m is roughly constant while the apparent maximum rate of nutrient uptake is inversely related to q, the cell quota (reviewed in Rhee 1979; DiToro 1979; cf. also Rhee 1973; Fuhs et al. 1972). Burmaster and Chisholm (1979) have, however, recently shown that V is only very weakly (if at all) related to the preconditioning growth rate. The success of the Droop formulation is dependent on the relationship between μ and the ratio of the minimum to the maximum cell quota for any particular limiting nutrient (Goldman and McCarthy 1978). It should be remembered that Droop (1975) himself points out that his model is "no more than an empirical description of algal growth" and does not imply any physiological mechanism. There are conditions for which the Droop formulation does not work well (cf. references in Goldman and McCarthy 1978); for example the effect of temperature has an independent effect on the cell quota of nutrients (Goldman 1979b).

In a perturbed system therefore nutrient uptake is uncoupled from growth in the short term and K_m and K_s differ markedly (Caperon and Meyer 1972a, b; Eppley and Renger 1974; Eppley and Thomas 1969). V is controlled both by the external and the internal nutrient concentrations. Recent perturbation experiments using nitrogen and silica have demonstrated this fact clearly (Collos 1979a, b; Conway and Harrison 1977; Conway et al. 1976; Eppley and Renger 1974; McCarthy and Goldman 1979). Nitrate assimilation is metabolically regulated (Bates 1976; Eppley and Coatsworth 1968; Falkowski and Stone 1975; Solomonsen and Spehar 1977). A similar situation exists for phosphorus uptake (Brown et al. 1978; Brown and Harris 1978; Chen 1974; Lean 1973a, b, 1975; Lean et al. 1975; Lean and Nalewajko 1976; Minear 1975; Nalewajko and Lean 1977; Norman and Sager 1978; Okino 1973; Perry 1976; Taft et al. 1975). "Luxury" uptake and storage of phosphorus may occur (Fitzgerald and Nelson 1966). This was observed some time ago by Mackereth (1953), in a paper which appears to have been largely overlooked. (Mackereth's (1953) comments about phosphorus limitation in lakes are also worth reading!) Errors in analytical techniques also appear to be significant at very low external nutrient concentrations (Brown et al. 1978; Lean 1973a, b; McCarthy and Goldman 1979; Rigler 1966). Some recent models of nutrient uptake by phytoplankton have incorporated the transient uptake kinetics (Davis et al. 1978; Lehman et al. 1975; Norman and Sager 1978; Nyholm 1978a) and have generated a certain amount of controversy (Davis and Harrison 1978; Droop 1978; Burmaster 1979b).

What the physiology literature now reveals is a whole suite of physiological responses in algae which serve to buffer the growth rate of the cell against fluctuations in the external environment. Such mechanisms have been revealed in fluorescence responses (Harris 1979a; Heaney 1978; Kiefer 1973a, b; Loftus and Seliger

1975), oxygen evolution (Harris and Piccinin 1977; Marra 1978a, b), carbon metabolism (Harris 1978), ammonium uptake (Conway and Harrison 1977), nitrate uptake (Caperon and Meyer 1972a, b), phosphorus uptake (Burmaster 1979a, b; Lean 1973a, b; Taft et al. 1975), and silica uptake (Davis et al. 1978). Such mechanisms have presumably evolved in response to fluctuations in the external environment. Recent work on Chl_a synthesis also shows rapid response to changes in light levels (reviewed in Harris 1978 but cf. more recently Falkowski et al. 1978; Hitchcock 1979a, b; Riper et al. 1979) (Fig. 1). For a time I wondered why phytoplankton would expend energy on rapid chlorophyll synthesis and destruction so that the chlorophyll pool has an apparent turnover time of 8–12 h (Riper et al. 1979). Evidenty, the cells have responded to an environment in which the incident irradiance changes on a diurnal and a daily basis. The mechanism has evolved in response to the fluctuating environment.

Methodology, Physiology, and the Natural Environment

From now on we shall have to characterize not only the time constants of the physiological process under study but also the time constants of the methodology and of the environment from which the cell was taken. Unless the time constants of the physiology, methodology, and environment all match one another, then the results and the understanding obtained will be imperfect at best. This is not a new idea; the problem was raised in an oceanographic context by Stommel (1963) (cf. also Haury et al. 1978). Some immediate problems are evident. Is it correct to use IVF which responds to the light climate very rapidly, to infer spatial distributions of chlorophyll which respond to light and growth at different scales (Fig. 1)?

Is it correct to use 2–8-h nutrient uptake and productivity measurement techniques? The original rationale for such measurements was clear: 2–8 h was needed to obtain a measurable response (cf. Talling 1973). Thus (with the exception of certain diurnal phenomena — reviewed by Harris 1978; Sournia 1974) the role of processes with time constants of hours and minutes was underestimated and poorly understood. New techniques (such as O_2 electrodes, Harris and Piccinin (1977); Harris (1978)) have improved the temporal resolution of productivity measurements. I have already mentioned the continuous culture perturbation experiments. It is now certain that the algae respond rapidly to fluctuations in the surface irradiance and that, under mixed conditions, in situ bottle techniques underestimate the true integral productivity because the physical environment of the free-floating cells is modified when they are confined to bottles (Harris 1973a, b, 1978, 1979b; Harris and Lott 1973a, b; Harris and Piccinin 1977; Harris et al. 1979; Jewson and Wood 1975; Marra 1978a, b). The problem with the interpretation of in situ bottle data lies in the mismatch between the length of incubation (hours) and the time for the physiological response by the cells. The response time of the algae appears to match closely the characteristics of the physical environment from which the cells were taken (reviewed in Harris 1978).

Problems therefore arise when there is the possibility of a mismatch between the time constants of methodology and physiology. Problems may also arise when a single method is used to obtain information about a number of algal processes which operate over a variety of time scales. An example of this is the recent tendency to use the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) with a fluorescence technique as an indicator of photosynthetic capacity, P_{max} (Samuelsson and Öquist 1977; Samuelsson et al. 1978). The original report (Samuelsson and Öquist 1977) concerned the use of the method on cultured material but since then it has been used in a variety of natural situations (Cullen and Renger 1979; Esias 1978; Harris 1979a; Kimmel and White 1979). The results from the use of the DCMU/fluorescence technique in the field have been, to say the least, inconclusive. Some report good results (i.e. a correlation between DCMU-stimulated fluorescence and photosynthetic capacity) (Kimmel and White 1979), others variable results (Cullen and Renger 1979), and yet others no correlation at all (Harris 1979a). Part of the problem lies in methodology (cf. discussion in Harris 1979a; Harris et al. 1979c) as the Turner or Turner Design fluorometers normally used have a much lower excitation energy than that used by Samuelsson and Öquist (1977), and it is not usual (in the field) to integrate the first few seconds of the response as did Samuelsson and Öquist (significantly Kimmel and White (1979) did this). The major problem, however, lies in the fact that the IVF response of cells (with and without DCMU) responds rapidly (minutes) to the previous light history of the cells (Harris 1979a; Harris et al. 1979c) whereas adaptive changes in the photosynthetic response of the cells take place at a number of time scales (reviewed in Harris 1978; cf. Steeman-Nielsen and Park 1964; Harris et al. 1979b; Jones 1978) (Fig. 1). Thus in a nonsteady-state environment the attempt to correlate IVF with P_{max} may not give meaningful results. Once again it is not possible to extrapolate from steady-state irradiances in culture to the field situation.

Thus there are methodological, physiological, and environmental problems with kinetic measurements in the field. I have recently discussed the relationship between methodology, productivity measurements, and growth at some length (Harris 1978, 1979b) but it is clear that the cells have a means to integrate the fluctuations in the external environment so as to produce as constant and as rapid a growth rate as possible. It is very difficult to extrapolate from kinetic measurements to in situ growth rates. We do not as yet fully under-

stand the relationships between our short-term uptake measurements and the realized rate of cell division in situ.

The Question of in situ Growth Rates

With the degree of uncertainty over the actual concentration of supposed limiting nutrients in surface waters and the problem of measuring growth rates in situ, it is hardly surprising that there is no apparent correlation between nutrient levels and growth rates (cf. summary of available data for marine waters in Goldman et al. 1979). When an algal cell only needs a brief period of nutrient supply to satisfy its needs for cell division, then the knowledge of the spatial and temporal scales of nutrient available becomes critical (Allen 1977; Goldman et al. 1979; McCarthy and Goldman 1979). If the nutrient is available as a species of small rapid pulses from sources such as entrainment or zooplankton excretion and/or bacterial activity, then the actual measured concentration may well be below detection limits, but the flux of nutrients continues. This had led Goldman et al. (1979) to question the whole concept of limiting nutrients in planktonic systems. Small-scale perturbations invalidate the steady-state approach. McCarthy and Goldman (1979) and Goldman et al. (1979) have recently suggested that such a pulsed nutrient supply might allow oceanic phytoplankton to grow at close to their maximum rates in nutrient poor waters (cf. Sheldon and Sutcliffe 1978). Part of the evidence comes from the stoichiometric ratios of C:N:P observed in oceanic phytoplankton which compare well with phytoplankton growing at near maximal rates in culture (Goldman et al. 1979).

The problem of the importance of small-scale, high frequency patchiness raises the question of the regeneration of nutrients within the surface mixed layer of lakes and oceans. Dugdale and Goering (1967), Dugdale (1976), and Eppley et al. (1979) have discussed the problem of defining what is "new" production and what is regenerated in the oceans. Basically the overall biomass ("size of wheel") of algae, zooplankton, and fish must be related to the total nutrient stocks available, but the rate of productivity (or the "rate at which the wheel is spinning") may vary over wide limits. This is by no means a new idea but it is one which appears to need restating occasionally, particularly in the context of eutrophication (Schindler and Lean 1974).

The phosphorus input/chlorophyll models of lakes (cf. references below) essentially assume that lake ecosystems process their nutrient inputs in similar ways so that, of the phosphorus added, so much ends up in the algae, so much in the zooplankton, and so on. That there is an overall relationship between phosphorus input and the standing stock (yield) of algae in different lakes shows that this must be so. There is, however, for any given input of phosphorus, up to an order of magnitude variability in the actual volumetric chlorophyll observed. This may be related to how the total phosphorus input is partitioned between the standing stocks of algae, zooplankton, and fish (Shapiro 1977, 1978; Shapiro et al. 1975), or to the hydraulic characteristics of the basin (Chapra and Tarapchak 1976). R. A. Vollenweider (Canada Centre for Inland Waters, Burlington, Ont., personal communication) has pointed out that some of the most interesting lakes are the ones which do not fit on the usual log–log plot — the partitioning of phosphorus in those lakes is modified for some reason.

These nutrient input/biomass relationships do not give any information on growth rates or productivity per se. While Schindler (1978) reports a significant relationship between net primary production and total phosphorus input for a number of (mostly temperate) lakes, the relationship is weaker than the biomass relationship and gives a spread of at least two orders of magnitude for any given phosphorus input. Thus, once again, the rate at which the wheel is spinning is known with a good deal less confidence than the ultimate size of the overall algal yield. As with community structures, the biomass distribution is known with some confidence but the rate processes are known with less confidence. Growth rates in situ are likely to be controlled by a host of factors acting in concert, but *given enough time* the overall yield will be a function of how the most limiting factor is partitioned between the biota.

In a hierarchical sense (Fig. 1) we have high-level models for the steady-state distributions and nutrient responses of phytoplankton biomass but we are aware that the high-level explanations may be modified by higher frequency (lower-level) responses (cf. Simon 1973). Growth rates in situ remain an almost total mystery. The only well-documented examples of in situ growth rates come from those organisms which show phenomena such as phased division (Swift and Durbin 1972; Weiler and Chisholm 1976).

The Algal Scale

Allen's (1977) paper on the concept of scale in algal ecology addresses the whole interpretive problem; to quote from his paper "microscopic algae may be mistaken for plants that happen to be small." What has been suggested by recent research is that fact that, to the algae, small scales are very important and that we cannot merely assume that they are "small plants." Allen (1977) writes: "From a human perspective microscopic algae are the most distantly scaled organisms that can be observed in the field ..." and furthermore "The ecologist is likely to measure the environment at frequencies too long and at a grain size too large to correspond to the short frequencly, fine-grain algal actuality. The most sophisticated numerical analyses of field data will be of no avail."

As ecologists what are we to do? I maintain that a better understanding of algal biology and physiology — which emerged over the last few years — can now provide some pointers; not only to present misconceptions

but to further progress. So what are the algae doing? Slobodkin and Rapoport (1974) considered the properties of a well-adapted population in a fluctuating environment (cf. also Cohen and Parnas 1976; Calow and Jennings 1977). They suggested that organisms are required to be flexible in the way in which they respond to environmental perturbations but that such flexibility must be maintained without great cost to the organisms. But what is the best strategy? Slobodkin and Rapoport (1974) considered that the best evolutionary strategy would be to possess a "multi-linked response system which is a combination of hierarchical and direct response mechanisms." This is precisely what research on algal physiology has revealed (Fig. 1). An external perturbation sets into motion a whole series of responses. If the perturbation is sufficiently small or rapid, then purely physiological responses will take care of the stimulus. If, however, the stimulus is longer lasting or of greater magnitude, then the responses will go deeper and deeper (or higher and higher in the hierarchy) until ultimately growth rates and community structures will be affected (cf. Fig. 1 in Slobodkin and Rapoport 1974). Clearly the application of a continuous stimulus (as in continuous culture techniques) neither mimics the real world nor elicits the complete response.

Some examples may clarify the existence and importance of such response systems in algae. Fluctuations in incident irradiance (I_i) cause fluctuations in the rate of photosynthesis in algae as well as other responses in the longer term. The response is also decidedly nonlinear. Fluctuations in I_i below I_K (the saturation parameter of Talling 1957a, b) cause linear fluctuations in the rate of photosynthesis (Harris and Piccinin 1977). When I_i exceeds I_K, however, a complex set of metabolic reactions sets in and produces a hysteresis effect with a time/intensity dependent function (Harris 1978; Harris and Piccinin 1977; Marra 1978a, b; Setlik 1968). The speed with which the hysteresis effect appears and the magnitude of the difference in photosynthesis in rising and falling light regimes appears to be correlated with the environment from which the cells were taken (Harris 1973a, b, 1978). Populations taken from low temperatures in spring (but also deep mixed conditions (Harris (1973a)) showed marked hysteresis very rapidly when compared to warmer summer populations.

It is interesting to speculate that as biochemical pathways must transmit the light stimulus and convert it to fundamental responses in growth, the speed and efficiency with which the message is passed through the system may vary depending both on other external factors (in this case, temperature) as well as the adaptability of the organisms. Adaptation is the ability to cope efficiently with the external stimulus in context of other stimuli which may be fluctuating at quite different time periods (rapid light fluctuations–seasonal temperature fluctuations).

If the light stimulus alters over longer time periods (hours–days) the first response is altered chlorophyll synthesis over the first 6–24 h (cf. review by Harris 1978; Beardall and Morris 1976; Falkowski et al. 1978; Hitchcock 1979a, b; Riper et al. 1979) resulting in a change in the cellular chlorophyll content. If the change in cellular chlorophyll content cannot cope with the change in the light stimulus then a change in growth rate will occur (cf. Fig. 1). Temperature is one factor that regulates the speed and magnitude of the change in cellular chlorophyll (Hitchcock 1979a, b).

Nutrient uptake kinetics show similar temporal responses. I have already discussed the regulation of nutrient uptake by both internal and external nutrient levels. There are multilinked response systems in existence also. Collos (1979a, b) has examined the nitrate uptake kinetics of cells and has shown that a hysteresis effect exists depending on the previous nutrient history. Thus the K_m varies depending on previous conditions. Whether or not this hysteresis effect can also be correlated with the environment from which the cells were taken remains to be seen. There are certainly a complex series of links between the uptake and the metabolism of nitrate (Falkowski and Stone 1975), the elucidation of which are as much dependent on a good methodology as they are of an understanding of the underlying physiology (Collos 1979a, b; Collos and Slawyk 1977; Harrison et al. 1979). The existence of a complex link between nitrate availability and growth rate was revealed by Cunningham and Maas (1978), who showed that after a rapid change in nitrate availability the growth rate oscillated with a period of about 70 h. They found "a time lag between changes in intracellular nutrient content and corresponding fluctuations in cell division rate." As they subsequently point out, such behaviour is not predicted by any existing theoretical treatment of the system, but such behaviour is certainly in accordance with the model of Slobodkin and Rapoport (1974).

A final example concerns the ultimate expression of environmental change; changes in community structure. Allen et al. (1977) pointed out that as the planktonic environment is changing rapidly and the community structure changes in response to environmental changes but with a time lag, simple correlations between environment and community structure will rarely, if ever, exist. Allen et al. (1977) and Harris and Piccinin (1979) looked both at environmental changes and at the corresponding rate of community change. Allen et al. (1977) showed that there were discrete periods of oscillation in community structure which corresponded to certain phases of environmental change. Harris and Piccinin (1979) and Harris et al. (1979a, b, c) examined the changes in community structure which resulted from changes in a physical forcing mechanism. The rate of community change (and also incidentally the rate of change of some physiological parameters) was correlated to the rate of change of the physical environment. Communities were most stable during long periods of stable physics (complete mixing during fall

overturn) and least stable during periods of variable stratification (spring and summer).

A time lag of up to a few days appears to be a good description of the lag between the environmental change and the physiological response of the algae (Harris et al. 1979b) while the response of community change is longer. Significantly Jones (1978) found that a 5-d lag best described the adaptive responses of algae to fluctuating light in Lough Neagh. All of this would tend to suggest that such environmental fluctuations that occur at rapid time scales are being sensed by the organisms, and the fact that response mechanisms have evolved must mean that they are an important feature of the environment. Furthermore, the fact that the biomass distributions appear to vary most at large scales must mean that the small scale of rapid environmental fluctuations is being effectively smoothed out by the physiological buffering mechanisms.

This does not mean to say, however, that these small-scale or high frequency effects may be ignored. Different species may well be sensing different features of the environment in different ways so the temporal and spatial spectrum of fluctuations becomes a resource dimension in itself, which the species may exploit. The interaction of cell size, growth rate, and the physiological response of the alga with the spectrum of environmental fluctuations means that for each individual the realized growth rate will be a damped, time-lagged function of the environmental changes. Thus the alga is a living transfer function of the enviromnent. Such transfer functions may not be simple time averages of the environment and some recent work on periodic nutrient inputs and competition seems to confirm this (Turpin and Harrison 1979). The species composition of the phytoplankton community may be controlled in part by the high frequency components of the environment.

Thus the algae clearly show evidence of the type of behaviour predicted by the model of Slobodkin and Rapoport (1974). The widespread occurrence of such phenomena has, I believe, serious consequences for our modeling and management strategies. I will discuss these consequences in the next section.

Models

So far I have tried to make the case that small-scale, rapid events in planktonic systems play an important role in shaping planktonic communities and that there has, in the past, been a tendency to underestimate the effect of such events. This has, I believe, led to some unfortunate attempts at modeling and water quality management in aquatic systems.

We are now aware, I hope, that kinetic measurements give unreliable predictions, and that average biomass predictions are almost the only safe predictions we can make. Can we then predict the timing and magnitude of algal blooms? Can we identify the likely dominant species in advance? Despite the claims of the modeling fraternity I believe the answers to these questions are at present no, no, and no.

There are basically two types of models currently in use in aquatic systems. These models are essentially either empirically based models of the Vollenweider type (cf. Bloesch 1974; Deevey 1972; Dillon 1975; Dillon and Rigler 1974a, b; Hutchinson 1973; Oglesby and Schaffner 1978; Schaffner and Ogelsby 1978; Schindler 1978; Schindler and Fee 1974; Schindler and Lean 1974; Vallentyne 1974; Vollenweider and Dillon 1974) or they are kinetic, mass-balance models of the Riley et al. (1949) type with added features (for a review of early production models cf. Patten 1968). A partial list of modern mass balance type models and their derivatives would include Bierman (1976), Bierman and Dolan (1976), Canale and Squire (1976), Cloern (1978), Desormeau (1978), DiToro et al. (1971), Groden (1977), Halfon and Lam (1978), Ikeda and Adachi (1978), Imboden (1974), Imboden and Gächter (1978), Jørgensen (1976), Jørgensen et al. (1978), Kremer and Nixon (1978), Lam and Halfon (1978), Nyholm (1978b), Park (1978), Park et al. (1974, 1975, 1979), Scavia and Park (1976), Simons (1976), Snodgrass and O'Melia (1975), Steele (1958, 1974b), Thomann et al. (1974a, b, 1975, 1976, 1977), Walsh (1975), Walsh and Dugdale (1971), Youngberg (1977). There has also been recent compilations of models, classified by structure (Jørgensen 1979a, b; Swartzman and Bentley 1979).

The empirical models of Vollenweider do not give any information as to timing peak biomass of species. They merely state that over the long-term (scale of a year or years), the average expected chlorophyll *a* will be related to the available total phosphorus in the system. I have already noted that predictions of productivity (kinetics) based on regressions are less accurate than biomass predictions, and R. A. Vollenweider (personal communication) has noted that seasonal chlorophyll *a* regressions work less well than annual means (cf. Schindler et al. 1978). In other words at shorter and shorter temporal scales deviations from the expected relationship become larger and larger.

A word of caution at this point. That annual averages work implies averaging large temporal *and* spatial scales. At shorter times and smaller scales the relationships may not hold. Over a year the surface of a small lake is mixed many times over, so the relationship works very well in small lakes (Schindler et al. 1978). In larger lakes phytoplankton growth effects will become significant at scales >1 km so we would expect (and observe) inshore/offshore effects in larger lakes (Vollenweider et al. 1974). Coastal zones may trap nutrients (Welch and Lindell 1978) so the inshore/offshore loadings will be very different. All lakes do not have the same phosphorus/chlorophyll relationships (Schindler et al. 1978) and such relationships may be modified by physical forcing (Harris et al. 1979b) in coastal zones (Boyce 1974). The ecology of phyto-

plankton in mixing zones with nonequilibrium conditions existing at small scales is little understood and is crucial to many management concerns (cf. Harris et al. 1979a, b, c).

The problem of temporal scales is further complicated by long-term residence time effects (Chapra and Tarapchak 1976). Such long-term approaches to the predicted value may explain the fact that while some lakes fit the observed relationship (Ogelsby and Schaffner 1979), others, in which the loading has changed, do not (Reynolds 1978b). We do not know the response times of whole lake systems such as this.

The response time of the system to a changed loading is a function of the relative magnitude of the input compared to the natural, internal recycle mechanism (Golterman 1973). Thus the speed at which the "wheel" is spinning will affect the rate of response. The efficiency of phosphorus use (when expressed as chlorophyll produced per unit phosphorus) declines as the productivity of the lake increases (Reynolds 1978b; R. A. Vollenweider personal communication) so a given input of total phosphorus will have a bigger effect in a lake with low total phosphorus concentrations compared to the same input into a more productive lake. This may either be due to some intrinsic biological feature of algal growth in lakes of varying trophy, or it may be due to the changed relationship between inputs and the internal recycle mechanism (Golterman 1973).

A recent report by Vollenweider and Kerekes (1979) indicates that oligotrophic lakes recycle nutrients efficiently within the mixed layer so proportionally larger amounts of the input are lost in the outflow as compared to more eutrophic lakes in which proportionally more of the P input is lost to the sediments.

That there is, on average, a long-term relationship between phosphorus and chlorophyll is perhaps remarkable considering the complexity of the nutrient/phytoplankton/zooplankton system (Lane and Levins 1977). The spread on the plot of log total P vs. log chlorophyll is at least of an order of magnitude and clearly some of this is due to grazing factors (Shapiro 1977, 1978; Shapiro et al. 1975). In the context of the planktonic system, however, there are either some rules which govern the partitioning of the total P between the compartments or it is all a central limit theorem phenomenon. I have a feeling that it is a central limit theorem phenomenon (May 1974a, b). The time averaged result is a yield limitation, however (O'Brien 1972). O'Brien (1972) pointed out that the distinction between growth rate limitation and yield limitation is vital and that it is often easy to confuse the two. O'Brien showed that yield limitations can be brought about by a rapid growth rate limitation at low nutrient availability, but the culture analogy must not be used in the field.

Why do I not consider that the culture analogy applies in the field? The evidence for a single nutrient limiting algal growth is, in my opinion, rather poor. The spatial and temporal variability in the nutrient supply coupled with active grazing, destroys the steady-state argument and the Michaelis–Menten kinetic analogy does not hold when K_m and K_s differ (Goldman et al. 1979; McCarthy and Goldman 1979). The in situ evidence for nutrient-limited growth is also poor as neither the true in situ rate of growth nor flux of the supposed limiting nutrient is known with confidence (Goldman et al. 1979). Other published work has also cast doubt on the single rate-limiting nutrient theory ("contrary to expectations" — Schindler et al. 1971) — with the exception that under peak bloom conditions the nutrient supply may be temporarily depleted (Morris et al. 1971; Peterson et al. 1974; Serruya and Berman 1975; Sheldon et al. 1973; Yentsch et al. 1977). Certainly many natural populations have atomic ratios of cellular constituents which closely match the well-known "Redfield" ratio, a feature which may be interpreted as indicative of growth at near maximal rates (Donaghay et al. 1978; Goldman et al. 1979). We need to know a lot more about the spatial and temporal scales of the mechanisms which recycle nutrients within the planktonic community (Rigler 1975).

It is interesting to note that perhaps the best-documented case for nutrient limitations in situ appears to be that for silicon (Lund 1949, 1950, 1969; Lund et al. 1963; Paasche 1973; Schelske and Stoermer 1971; Titman 1976).

One further piece of evidence may be indicative of conditions in situ. The work by Lean (1973a, b) and Lean and Nalewajko (1976) indicates that unicellular algae excrete low molecular weight phosphorus compounds and finally reach an equilibrium with the external phosphorus concentration. Why would algae, which have a high affinity for phosphorus, also release the element from the cell? Given that evolution works, we would surely expect algae to have evolved rapid uptake and *retention* mechanisms if phosphorus were frequently in short supply. If they also excrete phosphorus then can we not reasonably assume that the cost of phosphorus excretion is small in relation to other requirements. Both Lean and Nalewajko (1976) and Sweazy et al. (1975) report that phosphorus *exchange* is a very important part of the overall flux; surely not the sort of behaviour one would predict for a mechanism which had evolved under conditions of low phosphorus availability. All the evidence appears to point to the fact that phytoplankton have evolved to utilize the available nutrients at the spatial and temporal scales at which they occur. Natural selection works — the steady-state paradigm requires revision and the range of observable dynamics must be revised to include high frequency, small-scale effects.

Do the kinetic mass–balance models have any realism or validity? Clearly there are problems and they fall into two types: problems associated with the biological assumptions which underly the model structure and problems with the model structure itself.

By now it must be obvious that I do not consider that the use of kinetic models as presently formulated has any great validity. I have already dealt with the

problems associated with the use of steady-state Michaelis–Menten type kinetics. In a spatially and temporally varying environment K_s is not a valid constant to use, neither can constant stoichiometry be assumed. The assumption of constant stoichiometry for instance requires a cell, respiring at night, to release nitrogen and phosphorus in proportion to the respired carbon — a quite unrealistic assumption as respiration is not the reverse of growth. The use of multiplicative kinetics justified by the suggestion that several limiting factors may be operating in concert (Baule 1918; Verduin 1964; cf. review by Rhee 1979) is not supported by the experimental evidence (cf. Droop 1977; Rhee 1974, 1978, 1979; Swartzman and Bentley 1979; and the competition experiments of Titman 1976 and Tilman 1977). Some recent models have been modified in this respect (Desormeau 1978).

The model formulations which are used to compute photosynthesis are those which conform to static in situ bottle chain results (Steele 1962); they do not take the high frequency spatial and temporal scales of light fluctuations into account (see discussion on methodology, above, and Swartzman and Bentley 1979). Some recent models do, however, include some longer-term light adaptation (Groden 1977) effects.

The assumptions of the use of Michaelis–Menten and Droop type formulations have recently been examined by Burmaster (1979a) and DiToro (1979). They conclude that at *steady-state* the formulations are equivalent. The time constant of nutrient uptake is shorter than the time constant for growth as DiToro (1979) asserts, but I cannot agree that it is safe to assume, therefore, that the cellular nutrient concentration is in equilibrium with the external nutrient supply, and that growth may be calculated by the Michaelis–Menten formulation accordingly. Certainly *at equilibrium* the Michaelis–Menten and Droop formulations are equivalent (see above) so Michaelis–Menten calculations (based on external nutrient concentrations only) do not require a knowledge of cell quotas. All the evidence now points to frequent nonequilibrium conditions on a scale of hours and days so that the previous history of the cells is a necessary piece of information. DiToro (1979) also points out that an assumption of equilibrium avoids the necessity to compute the algae for each box of a multisegmented model (with a consequent saving in time and cost) but others (Simons 1976) have concluded that spatial segmentation in large lakes is very important. For example, the seasonal cycle of chlorophyll *a* in Lake Ontario is different in different parts of the lake (Vollenweider et al. 1974).

The problem with the structure of the mass–balance models, particularly the larger ones with many boxes, lies in calibration and verification (Jørgensen 1979a, b; Lewis and Nir 1978), and Snodgrass (1979) has written ... "current models are not yet verified to predict the rate of recovery of a lake to control of phosphorus or to predict phytoplankton response to water quality management schemes ... due to the lack of appropriate field data." I believe the verification problem also is rooted in the type of biological models now in use. Some of the larger models are so complex that they do not produce unique solutions. Scavia (1979) has produced a reasonable fit to Lake Ontario IFYGL field data using two completely different sets of coefficients in the model. It also appears that the models are quite insensitive to some vital biological parameters (e.g. the photosynthesis:respiration ratio, Simons and Lam 1979) and that the basic structure determines the type of seasonal response. The model of Thomann et al. (1975, 1976) produces two phytoplankton blooms in L. Ontario for every simulated year. The actual seasonal cycle of phytoplankton in the lake varies from year to year and spatially within the lake (Snodgrass 1979; Vollenweider et al. 1974).

Clearly the complex mass–balance models lack biological realism at a number of scales and by Levins' (1968) modeling criteria they may achieve generality, but they lack both realism and precision. The predictions are therefore not to be trusted (cf. the comments by Morales (1972) and Stearns (1972)).

The basic problem with the plankton models is that they do not adequately represent the observed dynamics of planktonic systems; equilibrium is assumed where it does not exist and spatial and temporal scales are confused. At least the Vollenweider-type approach has generality and realism; the precision may be low but the model is valid and the predictions do work.

The Implications of a New Paradigm

The Biological Response to Variability in Time and Space

Phytoplankton are exposed to a variety of scales of spatial and temporal variability in their natural habitats. I have presented evidence that the cells have evolved mechanisms not only to cope with such variability but to actively exploit it. The environmental variability encountered in planktonic environments means that extrapolation from bottle or culture experiments is fraught with danger and that the concept of a single growth rate limiting nutrient is at best a crude approximation to the real situation. Evidently nutrient recycling mechanisms are of paramount importance to the planktonic community.

So how does all this environmental variability interact with the phytoplankton community? I have a suggestion to make which appears to go some way to explaining the observed phenomena. Suppose that the planktonic environment is varying at a number of temporal scales, i.e. there is a spectrum of frequencies in the natural environment. (Let us, for the sake of argument, think of the environment as a single resource spectrum — there is in reality one for each variable.) Phytoplankton will respond to this environmental spectrum in different ways depending on their own fundamental periodicities. I suggest that the basic period

for each cell is the generation time — the period over which the environment must be integrated while one cell grows and divides into two. I further suggest that the normal situation in a phytoplankton community is to have a variety of species of varying doubling times reading and exploiting the environmental spectrum in different ways. This is a different way of stating the concept of "contemporaneous disequilibrium." Each cell is in effect a transfer function of the environment, as the fluctuations in growth rate will be a damped, lagged function of the environment. It is well known that there is a spectrum of cell sizes in planktonic communities (Conover 1978; Kerr 1974a, b; Platt and Denman 1978; Sheldon and Parsons 1967; Sheldon et al. 1972) and that such a size spectrum is coupled to a spectrum of growth rates (cf. reviews by Banse 1976; Erberhardt 1969; Fenchel 1974). Thus it is possible that community structure is coupled to the environmental spectrum through a spectrum of cell sizes.

Such a mechanism would explain a number of observations — notably the increase in species diversity during periods of summer stratification as compared to periods of turnover (Lund 1964; Margalef 1968; Reynolds 1976, 1978a). Intuitively it is possible to envisage that the stratified period, with all the possibilities of ephemeral thermal structures and internal motions, has more spatial and temporal variability than periods of continuous mixing and turnover. In the system with which I am most familiar (Hamilton Harbour) species diversity rises during the stratified period (G. P. Harris unpublished data) and begins to fall rapidly within a week of the onset of turnover.

In such a framework the occurrence of monospecific blooms must be a peculiar circumstance and must mark the exploitation of an environmental spectrum with a narrow band of frequencies by a single species. It would appear that the occurrence of monospecific blooms is correlated with the occurrence of periods of low physical variability — at overturn (*Asterionella* in the English Lakes, Hughes and Lund 1962; Lund 1949, 1950; Lund et al. 1963; Mackereth 1953; Talling 1966) or during stable thermal periods (*Ceratium* and *Microcystis* in English Lakes, Reynolds 1975, 1976; Reynolds and Walsby 1975; Harris et al. 1979; Heaney 1976; George and Heaney 1978, and marine dinoflagellate blooms, Holligan and Harbour 1977; Eppley et al. 1968). Note that species in stable environments exhibit buoyancy control (Reynolds and Walsby 1975). When I said that Vollenweider's phosphorus loading models may work because of a central limit theorem effect, I was suggesting that in a physically variable mixed layer in summer, the available temporal and spatial niches may become filled with a spectrum of species of varying size. These phytoplankton will be grazed by a spectrum of different sized zooplankton and when all the spatial and temporal niches are filled the overall distribution of total phosphorus is as predicted. Depending on the way in which the environmental spectrum sets up in spring, then perturbations to the community structure may prevent all the niches being filled all the time so short-term variations may be observed.

I find it interesting to note that Reynolds (1978b) observed that some of the English Lakes have not responded to changes in phosphorus loadings in quite the way in which Vollenweider and others might predict. I wonder if this is in some way because these lakes have a marked tendency to support monospecific blooms and therefore the full expression of phosphorus compartmentation by many species is not seen.

Round (1971) talked about cardinal or shock points in the seasonal succession of species. These may be interpreted as periods of radical change in the environmental spectrum, with a presumed broadening of the frequency band in summer. There is definite relationship between environmental variability and community variability. Allen et al. (1977) showed this effect and showed also that the exact correlation of environmental and community parameters was not possible because of the lag effects. I have made the same points with reference to Hamilton Harbour (Harris and Piccinin 1979; Harris et al. 1979c) and a computer ordination of a year's data in Harris et al. (1979c) shows small week to week changes during the stratified period. As predicted by Round (1971) the largest changes occurred at the onset and breakdown of stratification.

In discussing the environmental spectrum the mean value must be considered as well as the amplitude, periodicity, and phase of the spectrum (Fig. 2). I have already shown that phytoplankton adapt to the mean value of the ratio of euphotic zone depth to the mixing depth (mean z_{eu}/z_m ratio, Harris 1978). This value changes seasonally as z_m changes and the phytoplankton respond to the change (Sverdrup 1953). Normally the ratio is low under deep mixing (large z_m) and diatoms prevail — in summer the ratio is larger as z_m is smaller and greens, blue-greens, and dinoflagellates prevail (Harris 1978). Thus if the mean value of the resource parameter exceeds the range of adaptability for any given set of species, community change may result. Talling's (1957a, b, 1960, 1961, 1970, 1971; Talling et al. 1973) definitive work on the underwater light climate is a good example of what happens when a major change in a resource occurs. The extremes occur either when the water column is optically deep and growth is suppressed by low light availability at times of turnover, or the algae are self-shaded and growth is limited when the maximum areal biomass is needed.

There are more subtle effects of resource availability, however, which depend on the amplitude, period, and phase of the components of the environment (Fig. 2). In Hamilton Harbour the z_{eu}/z_m ratio changes seasonally and the phytoplankton adapt (Harris 1978). During the summer, however, the z_{eu}/z_m ratio varies irregularly so that the value becomes essentially unpredictable for periods of time. These result in periods of reduced productivity by the algae (Harris et al. 1979b). Given the mechanisms which exist for coordinating the physiology and growth rates of algae, the exposure of cells to

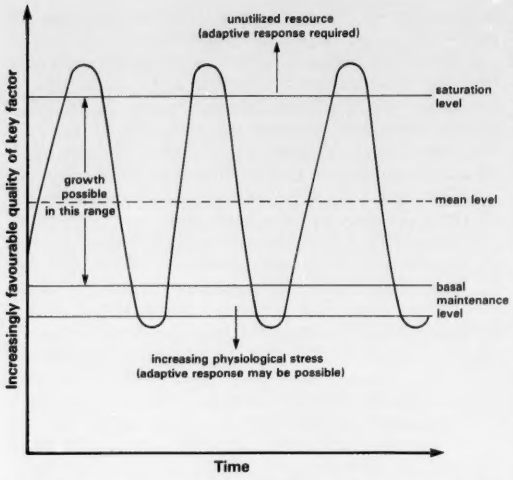

FIG. 2. A diagrammatic description of the presumed effects of a fluctuating resource on the performance of an algal cell. Variation in the mean level may bring about an adaptive response depending on the speed and magnitude of the change. The cell also responds to the amplitude, period, and phase of the variations, particularly if the resource availability frequently drops into or below the basal maintenance level. I am indebted to David Rollo for the original version of this figure.

a temporally unpredictable environment must represent a stressful situation (cf. May 1974b). Natural selection cannot operate in a regular way in an environment which shows no repeatable behaviour (Levins 1973; May 1974b). Thus we may expect that cells have evolved (in terms of their spatial and temporal constraints) to exploit certain features of the environmental spectrum and the environmental spectrum determines which species occur and when. The spectral approach to the description of the physics and biology of surface waters (reference above) does reveal some regularities in the partioning of variance in different systems. The spectra in Hamilton Harbour are much flatter than those observed elsewhere (Zarull 1979) so there is relatively more high frequency information in that system. The spectral approach to the structure of pelagic marine ecosystems shows some promise (Platt and Denman 1978) and should be compared to the distribution of variance in the environment.

The properties of the environmental spectrum also may be related to community structure through shifts in competition between species. Turpin and Harrison (1979) showed that the patchiness of a nutrient resource in time had a profound effect on the outcome of competition between two species of different sizes. The outcome of the competition was determined by the average level of the nutrient resource and by its temporal variability (Fig. 2). This may explain why species diversity decreases and productivity increases as the nutrient supply to the planktonic community rises (Margalef 1968). The mechanism of this effect really is the nub of the water quality management problem. As I have already said our present predictive capability is limited to a statistical reliance on long-term averages and the central limit theorem. This is not telling us the timing and magnitude of the expected blooms or the species composition of those blooms. Present models do not specify the real nature of the planktonic environment.

Consider Fig. 2, when the mean and the nature of the variability in the environmental spectrum are considered from the point of view of the cell, a knowledge of the following information is essential. How frequent and predictable will be the periods when the availability of the resource (P, N, or light, for example) is low and only basal maintenance will be possible? Temporal predictability is important if the cell is to harbor and partition its resources in a controlled fashion (Cohen and Parnas 1976). The frequency of such periods will interact with nutrient uptake rates and with the spectrum of cells, size and growth rates to produce the community structure. The periodicity and amplitude in the availability of (say) the nutrient will be largely controlled by recycle mechanisms. I have already discussed the fact that the response of the water body to external nutrient inputs will depend on the relative sizes of the input and the recycled supply (Golterman 1973). Oligotrophic to somewhat mesotrophic waters may be expected to show maximum diversity as they are entirely dependent on the recycled nutrients; the supply will be very patchy (Goldman et al. 1979; McCarthy and Goldman 1979) at small scales. Phosphorus utilization is very efficient (Reynolds 1978b) even though, largely because of grazing pressure, the biomass is low. Growth rates (Sheldon and Sutcliffe 1978) and grazing pressure may be high (Sheldon et al. 1972) in these circumstances. As external supplies are added, however, the mean level of the resource will be raised and the patchy recycle mechanisms will be relatively less important, i.e. the fluctuating resource will less often dip into the basal maintenance area (mean value increased or amplitude decreased, or both). As the patchy recycle mechanism becomes less important, species diversity should decrease even though productivity rises. Essentially we move towards K_s competition. Culture experiments must be equivalent to the ultimate eutrophic situation; no recycle and all constant external supply. As the species diversity drops we observe two correlated phenomema: the efficiency of phosphorus utilization decreases (Reynolds 1978b) as the uptake kinetics change toward K_s and the full gamut of spatial and temporal niches is no longer filled; and the recycle mechanism itself becomes less efficient as zooplankton shift from herbivory to detritus feeding (Gliwicz 1969, 1975; Gliwicz and Hillbricht-Ilkowska 1972). An increase in the mean nutrient level tends to favor larger cells because of the shift towards steady-state uptake kinetics

and because larger cells tend to have higher K_s values (Eppley et al. 1969; Parsons and Takahashi 1973). Larger cells will also alter the whole pattern of grazing in the community.

Such a set of occurences would explain the observations (noted above) that phosphorus or nitrogen limits growth only under bloom situations. As external nutrient supplies are increased, competition tends to favor reduced diversity and one or two (possibly larger) species may greatly increase in numbers. If a bloom is observed, grazing must be relatively less important so that the recycle mechanism is partially (or completely) disabled. This will result in a reduced internal nutrient flux, nutrient limitation of growth, and overall less-efficient nutrient utilization.

Phase information in an environmental spectrum may also be exploited. Perhaps one of the best examples lies in the vertical migratory behavior of dinoflagellates. Different species show different migratory strategies (Eppley et al. 1968; Heaney 1976; Kamykowski and Zentara 1977; Talling 1971) with some populations showing light compensating behavior (Harris et al. 1979). By using fluctuations in the environmental spectrum out of phase, the competition between two species may be reduced. In the *Ceratium* populations of Esthwaite water there may be different subpopulations within the lake, some of which move upwards during the day for light while others descend for nitrogen from the oxycline (George and Heaney 1978). Thus more efficient use of the environmental spectrum results. This may explain why *Ceratium* dominates the phytoplankton in the lake in summer.

Thus a recognition that steady-state kinetics do not describe the real world situation coupled with the realization of the importance of the spatial and temporal variability of the natural recycle mechanisms provides an explanation of community dynamics and also to some extent settles such arguments as those between Parsons and Takahashi (1973, 1974) and Hecky and Kilham (1974). The spectrum of cell sizes which occur responds to the dynamics of the environment in a very complex fashion. I believe that the basic community diversity (as a spectrum of species) is determined by intrinsic factors (basin morphometry, thermal structures and periodicities, seasonal climate, etc.) which may be modified to some extent by human external influence. Increased nutrient inputs from external sources should result in higher biomass and some reduction in diversity. Intrinsic factors may, however, play a part, as year-to-year differences in climate influence the environmental spectrum. Hamilton Harbour shows year-to-year differences in thermal structure, biomass, and diversity under constant loading which appear to support these arguments (G. P. Harris and B. B. Piccinin unpublished).

Management Implications

Management of water quality implies an ability to intervene, and an understanding of processes. The idea of management of water quality in a hierarchical ecological context implies an extra level — a level of control. It would appear that the main preoccupations of water quality management are large-scale and long-term. Scales of seasons, years, and lake basins seem to dominate thinking.

In a large lake the spatial scale is large enough for phytoplankton growth effects to occur in different parts of the lake (i.e. scales $\gg$1 km). From a water quality management point of view I do not believe that we can treat an area such as Lake Ontario as one homogeneous unit. Total phosphorus models may well work on a scale of years (Chapra 1977) but the biological components such as chlorophyll vary at much shorter scales. We must consider local, coastal effects as the mixing time in, say, Lake Ontario is much longer than the algal generation time. The basin is not biologically homogeneous (Vollenweider et al. 1974). The concept of a single growth limiting nutrient is a very good one — at steady-state or in culture. When applied at the whole ecosystem level in a spatially and temporally varying environment, the concept is meaningless. The human ability to intervene in ecosystem processes is limited to an ability to alter the extrinsic factors only. As a management tool it may suffice, but the control is limited to control of the biomass of the whole phytoplankton community as a result of large-scale statistical averaging. Species composition is dependent on intrinsic factors, which we can at present neither fully comprehend nor alter, although Shapiro (1977, 1978) and Shapiro et al. (1975), in advocating biomanipulation, probably have the right idea. Changing the herbivore populations would drastically alter the recycle mechanism.

The present controversy surrounding the evidence for long-term trends in the magnitude of the hypolimnetic oxygen deficit of Lake Erie (Charlton 1979) is a good example of our lack of knowledge. Long-term data are lacking, year-to-year climatological influences on the physical structure of the lake are poorly understood, and the ability of management techniques to control eutrophication can no longer be taken for granted. Removal of the external phosphorus load (short of reafforestation) may not control the hypolimnetic oxygen deficit in Lake Erie because of climatological variables. The utilization of phosphorus within the lake will depend on the phytoplankton species dynamics, niche packing, and the efficiency of the recycling mechanisms all of which are intrinsic features of the lake ecosystem. The oxygen depletion problem depends on a basin scale mass–balance and the algal dynamics. Control of algal dynamics alone will not suffice. The intrinsic biological features of the lake ecosystem have also been modified by a host of other changes besides those induced by eutrophication (Beeton 1969). We do not understand how such changes might interact with the effects of an increased nutrient supply (Lane and Levins 1977). We need long-term

data (cf. the Russell cycle in the English Channel), and the management of science must include a provision for such studies. The "today is Thursday, it must be lead" syndrome, with short-term projects carried out for customer organizations hinders or prevents the collection of long-term data sets. Personnel and methodology must not change from year-to-year or else long-term comparisons are useless. Stability in management personnel and funding is essential — this point has already been made recently in this journal (Vallentyne 1978).

We also require a change in water quality management thinking in such areas of the use of chlorophyll data and the use of the limiting nutrient concept. There is confusion concerning limits to growth and yields. Clearly the next stage is an attempt to predict the timing, the magnitude, and the species composition of summer blooms. I believe that real progress can be made as long as we stop thinking of all algae as a solution of pigment and we stop using overly simple concepts of nutrient availability. The biological reality must be faced if we are to make progress. I am fully aware that management personnel are often required to come up with "quick and dirty" answers in a hurry. What degree of control do we require, however? High level management concern (large-scale, long-term) may be achieved by a high level understanding but more and more it seems that we need to know something about the species themselves — be it for drinking water supplies in reservoirs or red tides in the oceans.

Sampling schedules need to be revised. For years we have been using a set of techniques with entirely the wrong resolution (cf. Stommel 1963). We need new sampling techniques at much smaller scales and new data. This is particularly true in mixing zones (such as coastal regions of the Great Lakes) where we know the physics are peculiar to such areas (Boyce 1974), that the spectra are flatter or altogether flat (Zarull 1979), and that the species respond to the large amount of high frequency information in the environment (Harris et al. 1979a, b, c; Harris and Piccinin 1979).

We certainly need more information on the relationships between external loadings and internal recycle mechanisms. Lakes are ecosystems — so are the oceans. They process materials and recycle them very rapidly and the response of the systems depends as much on the intrinsic properties as it does on the inputs. Once again we lack basic biological information. I recommend that N. America pay less heed to the creed of systems engineering and move to the (European) tradition of basic biology. Let the large-scale models wait until we have the necessary biological skills to model, process, and pattern at realistic scales. Computer technology exists in excess at present — is management prepared to bide its time until the biological work is done? — I fear not. Let those who use the results of models be wary of simplistic predictions.

So where do we go from here? Evidently we need to know a lot more about the spatial and temporal variability in the planktonic environment — viewed from the point of view of the alga. *Scale is important* (Allen 1977). The study of the grazing process appears to be vital to an understanding of how the phytoplankton community is set up together with more information on the role of microscale patchiness. The time series approach to the study of phytoplankton ecology may be a fruitful avenue of enquiry. If nothing else we need to know how patchiness in space and time and species assemblages are related. Certainly a revision of the standard paradigm is required.

From the perspective of modeling and management, a new generation of models is required. We need realistic models of species structure if we are to predict the timing, magnitude, and species composition of algal blooms. I believe that progress can be made along the lines I have outlined above.

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Comments on the Analysis and Sampling of Low Conductivity Natural Waters for Alkalinity

Numerous research programs are now being developed in Ontario and elsewhere in eastern Canada to study the effects of acidic precipitation on aquatic ecosystems. Scientists in this field have identified a major need in this regard; that is, to develop a comprehensive and accurate inventory of lake and stream water buffering capacities which may then

a) be used to show which geographic areas are most susceptible to acidification, and
b) in conjunction with models developed from detailed lake studies, be used to predict the future effects of continued input of acidic precipitation on these water bodies.

Since it is beyond the capability of any one agency to collect the data adequately for the inventory, meeting the objective will require a cooperative effort and an agreement to use methodologies which are both compatible and will yield as precise information as possible.

With regard to data precision, application of many traditional techniques (which were originally developed for waters or wastewaters with high inorganic carbon) is unacceptable. In particular, the precise measurement of alkalinity requires special care and methodology when analyzing the typically dilute waters found on the Canadian Shield. This fact was recognized by Schindler and Nighswander (1970), who employed appropriate techniques while investigating Clear Lake (Haliburton). Their small data set constitutes the best historical alkalinity values available for Ontario at this time.

Most of the present water quality data base in Ontario contains alkalinity values determined by the "total fixed endpoint" (TFE) procedure (Dillon et al. 1978) and usually expressed as $mg \cdot L^{-1}$ $CaCO_3$ (units of $meq \cdot L^{-1}$ are probably less misleading and more useful to scientists considering the depletion of alkalinity in freshwater systems). This method assumes that the end point of the titration occurs at pH = 4.5, defined either potentiometrically or with a bromocresol green-methyl orange indicator. In waters which have a high total inorganic carbon content (C_t) and therefore are highly buffered, the end point (corresponding to the point of $[HCO_3^-] - [H^+]$ equivalence) is close to pH = 4.5 (e.g. for $-\log C_t = 2.5$ or $\simeq 40\ mg \cdot L^{-1}$, end point pH $\simeq$ 4.45; Fig. 1). The relative error introduced by assuming a fixed end point pH is unimportant in this case. In waters with lower C_t's the titration end point shifts towards higher pH values (e.g. for $-\log C_t = 3.5$ or $\simeq 4\ mg \cdot L^{-1}$, end point pH $\simeq$ 5.00; Fig. 1) and hence the TFE procedure overestimates the "true" alkalinity or "total inflection point" (TIP) value. Although a thermodynamic relationship exists between them, a mathematical correction of historical data bases is not possible. The TFE data tend to differ from the TIP values in a near-random manner due to the large indeterminant error in TFE inherent in attempting to define the end point near or at the position of greatest change in slope of the titration curve. The TIP datum is best determined using a Gran analysis, a procedure which linearizes a buffered portion of the curve (discussed by Stumm and Morgan 1970).

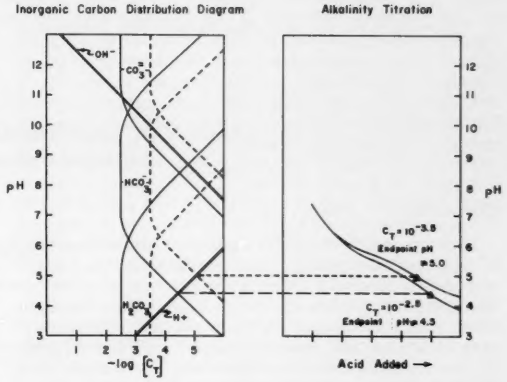

FIG. 1. Idealized theoretical relationship of inorganic carbon species to the alkalinity titration curve. The end point of the titration (▲) corresponds to the $[HCO_3^-] - [H^+]$ equivalence point. Note that the units of titrant addition for the alkalinity titration are arbitrary and different for each of the curves (adapted from Stumm and Morgan 1970).

In addition to the limitations imposed by the imprecision of the TFE methodology, a second source of error may exist if water quality samples are collected at the surface of a lake. Many workers have observed large variations in pH and inorganic carbon in surface waters, presumably due to the influence of primary production. In such situations, a surface sample is clearly not representative of the whole lake. A volume-weighted sampling procedure provides the most representative lake aliquot, although collection of an integrated column sample using a flexible plastic tube lowered to the bottom of the metalimnion is a simpler acceptable alternative.

In summary, we emphasize the importance of developing a large, precise inventory of buffering capacity information for freshwater in eastern Canada. To achieve this objective, the following actions are prerequisite:

a) scientific cooperation and communication among the diverse groups and agencies who are or have been actively involved in data collection,

b) use of TIP analytical methodology (i.e. Gran analysis) which will provide data of acceptable precision, and

c) use of sampling procedures which will yield as representative whole-lake samples as possible (surface samples are generally unacceptable).

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Methods of analyses and sampling have been detailed in Ontario Ministry of the Environment (1979). — DEAN S. JEFFRIES, *Limnology and Toxicity Section, Ontario Ministry of the Environment, Box 213, Rexdale, Ont.* and ANN P. ZIMMERMAN, *Department of Zoology, University of Toronto, Toronto, Ont.*

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Comment on "Quantitative Rights as an Instrument for Regulating Commercial Fisheries"

COMMENT

Moloney and Pearse (1979) provide a useful description of a "quantitative rights" system for the economic rationalization of commercial fisheries. However, they present an incorrect interpretation of a very similar system that I proposed several years ago (Christy 1973)[1] and make no reference to similar ideas proposed by others (Bell and Fullenbaum 1973; Mueller and Vidaeus 1978). Because their proposal discusses what they refer to as a "novel" idea, it may be worthwhile to clarify the record.

> With regard to my proposal, they state that:
> it has several features that we consider disadvantageous and hence distinguish it from the system described below. One of these is that the quotas are defined as a percentage of the total catch, which would restrict flexibility in their allocation and add to the difficulty of adjusting the catch downward. More important, Christy's quotas are not transferable, although, as we explain below, transferability serves a valuable economic and administrative function.

Although it is true that I proposed that the quotas should be expressed as percentages, it is not clear that this reduces flexibility or increases the difficulty of downward adjustments. The difference in the two approaches lies not so much in whether the shares are defined in terms of percentages or in terms of pounds or tons, as it does in the degree of power that the management agency can exercise in the market for quotas. Under the Moloney and Pearse approach, the desired flexibility can be obtained only if "the authority could stand ready to enter the market for rights, buying or selling at the market price in response to changing management requirements." Under my approach, flexibility in response to changing requirements in total allowable catch would be automatic, but in the case of a downward adjustment, each shareholder would be required to accept a smaller quantity of catch if the agency is unable to buy up a sufficient amount of shares.

The essential difference is that under their approach, the agency *must* take up the slack in a downward adjustment, whereas under my suggested approach, either the agency takes up the slack or the fishermen share in the decreased yields (as occurs generally under present conditions). I suspect that the difference in our approaches reflects the difference in the degree of authority that can be exercised by, and the amount of funds available to, Canadian and American management agencies.

Their second point is more serious but less valid. Although my proposal places a restriction on the transferability of quotas, it certainly does not prohibit transferability. Indeed, as I have stated elsewhere in describing this proposal, "it would be essential that the quotas be transferable — that the fishermen could lease or sell their quotas to others or to the administering agency" (Christy 1975). In my initial presentation, I suggested a free market in the leasing of quotas among fishermen, but with a restricion on the sale of quotas by providing the management agency with the sole right of purchase. This admittedly restricts the rights associated with the fishermen's quotas, but the restriction certainly does not make the rights nontransferable, as is stated by Moloney and Pearse.

In a legal sense, it would appear that under the proposal by Moloney and Pearse, the essential property rights appertain to the fishermen, whereas under my proposal the essential property rights are vested in the hands of the public and exercised through a public agency. In the latter case, the quotas would have more of the attributes of a permit than of a property right. They would be analogous to the grazing permits available for some of the public lands in the western United States.

In a functional sense, the rationale for restricting the purchase and sale of quotas to the agency is to facilitate management of the resource. With this restriction, the agency would presumably be able to acquire shares of the yield more easily than under the system suggested by Moloney and Pearse.

From society's point of view, a case might be made that the resources are part of the public domain and that the property rights should properly be in the hands of the public.

Each of these points is debatable, and arguments can be presented both for and against placing a limitation on the rights of sale. It seems to me that it would have been better for Moloney and Pearse to have discussed the advantages and disadvantages of the different features in our proposals than to have discarded mine on the mistaken interpretation that my proposed quotas are nontransferable.

[1]This paper was incorrectly referenced in both the Scott and Crutchfield articles which appeared in the same volume as the Moloney and Pearse article.

— FRANCIS T. CHRISTY JR., *FIPP Fisheries Department, UN Food and Agriculture Organization, Via delle Terme di Caracalla, Rome 00100, Italy.*

BELL, F. W., AND R. F. FULLENBAUM. 1973. The American lobster fishery: economic analysis of alternative management strategies. Mar. Fish. Rev. Pap. 994.

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REPLY

Francis Christy raises two issues that deserve further comment. One is the matter of transferability; in view of the apparent confusion about both the meaning and implications of this important property of resource exploitation rights some clarification will be helpful. The verb "to transfer" means (according to Merriam–Webster) "the making over of a right, title, property, or the like, from one person to another"; and in our article to which Christy refers, we have accordingly used the term "transferable" to describe rights or licences that can be made over from one party to another (Moloney and Pearse 1979).

Christy, in his 1973 discussion paper, proposes that the sale of fishermen's quotas "... would not be permitted except to the administering agency" and beyond this that holders would be allowed only to "lease" their quotas to others, possibly with limits on even these arrangements (Christy 1973, p. 2). In his subsequent conference paper he contemplates quota sales among fishermen, but again suggests the desirability of limiting exchanges among fishermen to "leasing" and requiring "... that all sales of quotas be made to the management agency" (Christy 1975, p. 98–99). This implies, at most, a very restrictive definition of transferability, and explicitly not the free marketability of rights which we consider to be important.

In another recent article on the subject, Frank Meany argues that "Theoretically a fishing boat licence cannot be sold. It can only be transferred with the permission of the licencing authority" (Meany 1979, p. xii, fn.). This involves further misunderstanding, because although the legal sale of anything must be permitted by the government in a general sense, whether explicit governmental permission for the transfer of a resource right is required depends on the terms and conditions of the particular rights.

The transferability of rights is extremely important to the efficiency and administration of a fisheries regulation policy based on quantitative rights, and we have advocated free transferability and divisibility of these rights rather like the free marketability of shares in the stock market or perhaps more akin to futures in a commodity market. This will serve three valuable and related functions which cannot otherwise be easily served. In the first place, it will ensure that the fishing rights will be reallocated, through voluntary sales and purchases, to those fishermen who can take the catch most efficiently, because they will be able to offer the highest price. In the second place, it will permit each owner of a fishing enterprise to acquire, with certainty, rights to the quantity of fish that can be harvested most efficiently by his fishing unit, and at the same time to adjust the size of his unit to the most efficient scale in light of current fishing technology. In the third place, as we have explained, the behavior of fishermen "... depends on the price of catch rights, and his choice of optimal scale therefore requires that the price of rights accurately reflects the social value of the resource" (p. 862) and that this correct price will be struck by a freely competitive market. Obviously, if the government were in the position of a monopsonistic buyer, the price of rights would be arbitrary.

Moreover, these functions cannot be served equally well by provisions for leasing quotas, as Christy proposes, particularly if adjustments to vessels and to the fleet needed to accommodate changing conditions can be made only with some time lag (i.e. if capital is "non-malleable"; Clark et al. (1979)). This or other imperfections would prevent the leasing charge from reflecting the changing value of the resource rent.

The other issue Christy raises is whether it is more advantageous to denote rights in terms of a percentage share of the total allowable catch, as he suggests, or in terms of absolute quantities of fish, as we suggest. Clearly, it makes no difference if the total allocated catch was constant from one season to the next, so the issue is how best to accommodate the need for variations. We favor absolute quantities for both efficiency and administrative reasons. An important determinant of the efficiency with which an enterprise can plan its activities is the certainty of its access to resources. Quantitative rights expressed as quantities will clearly afford greater certainty and security than rights that provide only a fraction of a variable total. Incidentally, the market price of an uncertain quantity will be less (even if it provides the same amount in the long run) because it will be discounted as a result of its unpredictable allocation over time.

Christy emphasizes administrative flexibility, and suggests that a percentage quota would facilitate downward adjustments because each share would be automatically adjusted in light of an alteration in the allowable catch. As a practical matter, we question this. Experience with various kinds of resource allocation systems indicates that regulatory agencies always find it difficult to reduce rights below the level that users have previously enjoyed; timber quotas, water rights, grazing rights, and allocations under international fisheries conventions all provide evidence of the difficulties involved in reducing the privileges of resource users, especially at short notice.

In contrast, the quantitative rights we propose would not call for any involuntary reduction in users' allocations. It is true that if the total long-term average allowable catch were fully allocated under continuous quantitative rights, the administering authority would have to purchase or sell rights in most years to effect required adjustments (much like a central bank's open market operations in bond markets and foreign exchange markets). However, we sus-

pect that a more practicable way of providing for adjustments to the catch of fluctuating stocks would be to issue basic rights which, in total, amount to roughly the minimum expected allowable catch, and then to auction or otherwise issue supplemental one-season rights at the beginning of or during each season to make up the difference between the sum of the basic rights and the allowable catch for the current season.

We should point out, however, that neither we nor Christy have adequately examined the implications of these alternative arrangements in terms of their administrative complexity, fairness, flexibility, and the powers they confer on bureaucrats. We tend to favor systems based on market mechanisms rather than compulsion, but the relative merits of our proposals in these respects need much fuller investigation.

Christy makes no reference to other literature in either of his papers, but the article by Bell and Fullenbaum he mentions in his comment briefly discusses a system based on catch rights allocated to fishermen, among alternative approaches to regulating the American lobster fishery. Their "stock certificates" are freely tradable, although it is not clear whether they are meant to be denoted in absolute quantities or percentages (Bell and Fullenbaum 1973). (The reference to Mueller and Vidaeus appears to be a memorandum to which we do not have access.) But these are only incidental contributions to a now considerable literature, extending over the last two decades; for a critical review, readers should refer to the recent paper by Anthony Scott (1979). We described quantitative rights as being "novel" not because they are new to the literature but rather because of our dearth of experience with them as instruments of fisheries management.

It is timely to debate these and other aspects of possible regulatory arrangements involving quantitative rights. In the last couple of years we have seen the first experiments with this approach to regulating fisheries. In the shrimp fishery off the coast of Labrador the Canadian government has recently introduced a licencing system that provides each licensee with the right to catch a fixed number of tons, and a similar arrangement is being considered for the scallop fishery on Georges Bank. Licenced herring fishermen in the Bay of Fundy recently organized themselves into a "club" through which the allowable catch is allocated among the members as vessel quotas. On Canada's Pacific coast the small abalone and herring roe-on-kelp fisheries are also regulated in this way. And a couple of months ago Italy introduced a new management system for its clam fishery which involves a quota for each licensee. There are probably other examples, and more to come. We suspect that experience under these experiments will reveal significant advantages of this general approach to regulating fisheries over the more familiar alternatives. — D. G. MOLONEY AND P. H. PEARSE, *Department of Economics, University of British Columbia, 2075 Westbrook Mall, Vancouver, B.C. V6T 1W5.*

BELL, F. W., AND R. F. FULLENBAUM. 1973. The American lobster fishery: economic analysis of alternative management strategies. Mar. Fish. Rev. Pap. 994.

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BOOK REVIEWS/COMPTES RENDUS

Book review editor:
David P. Scott
Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

Biology of Intertidal Animals. 3rd Edition. *R. C. Newell. Marine Ecological Surveys Ltd., Faversham, Kent, U.K. 1979. ix + 781 p. £22.00.*

The extent to which intertidal animals can achieve a net energy gain from the environment represents the energy which is available for growth and reproduction and is a measure of the ability of the organism to compete with neighboring species with similar tolerances of physico-chemical conditions. The first major revision and expansion of the original text (1970) focuses on the main components of the energy balance equation:

net assimilated energy	=	food consumed	−	losses through feces, dissolved organic matter, and respiration	=	energy available for growth and reproduction

Although it does not deal in-depth with growth and reproduction, i.e. the ways in which organisms use this net energy gain to best competitive advantage, it does take us substantially closer to explaining the distributions and abundancies of intertidal animals.

The first edition was an attempt to account for the distribution of intertidal animals in terms of physiological responses to the complex array of environmental factors by identifying environmental factors or sets of factors responsible for limiting a species. Problems with that approach were the difficulty of understanding the ecological significance of single factor experiments or of interpreting interactions between factors in multifactor experiments. In any case, a complete description of the environment experienced by a population is rarely known. It is now clear that intertidal organisms, except some living high in the intertidal zone, live well within their limits of tolerance to the environmental factors.

The use of the energy balance concept provides a point of impingement of numerous fields which bear on the distribution and abundance of intertidal organisms but which are rarely brought together in one work: classical analysis of intertidal zonation, physico-chemical nature of the intertidal environment, adaptive physiological response, behavior, intermediary metabolism and biochemical adaptations, functional morphology, and energy flow.

The book is essentially divided into two sections, the first dealing with physical, chemical, and biological features of the intertidal zone and the establishment and maintenance of distribution patterns. The remainder of the book deals with energy acquisition (principally food resources and feeding), with metabolic energy expenditure (principally respiration, gas exchange, transport, and metabolism) and with the balance between the two. Much of the original text is repeated unchanged, though often reorganized in the third edition. The new material adds to the book in three ways. It expands the treatment of deposit shores. For example, in the chapter on the faunas of special habitats, an extensive section has been added on the interstitial fauna of intertidal deposits, its nature, distribution horizontally and vertically and its adaptations. The fauna of subsaline salt-marsh pools is also described in that chapter. The discussion of the modification of sediments by biota has been expanded. Secondly, material on tropical and subtropical organisms and shores has been added, for example, on the behaviors of the surf clam *Donax*, and the crabs *Ocypode*, *Uca*, and *Emerita* in maintaining their positions on the shore, and on heat relations of tropical gastropods. Thirdly, the bulk of new material added is the literature of the 1970s such as discussions of the fragmentation and heterotrophic biodegradation of coastal macrophytes (kelps, eel grass, salt-marsh grasses, mangroves) to form an important food resource to intertidal animals; the nutritional importance of microorganisms and the partitioning of energy on the shore. Another major updated area is the treatment of tolerance of environmental stress which concludes that limits of tolerance are best described in terms of multivariable response surfaces and that these have ecological significance primarily for animals living high on the shore or for interstitial organisms. A new chapter considers strategies for compensating for environmental changes by adjustment of feeding rate, or metabolic energy expenditure or both.

The topics that are covered by the book are handled in a broad, thorough and very readable manner. Nevertheless, the book is not yet a comprehensive treatment of the biology of intertidal animals. Several major areas receive scant attention, such as the energetics of digestion, excretion, growth, and reproduction. Other topics such as osmotic and ionic regulation are treated only as they affect oxygen consumption. As it stands, the work does not permit us to test fully the usefulness of energy balance in explaining species abundances and distribution and community structure in the intertidal zone.

The third edition is 50% longer than the original text; cited references are increased to about 1825 from just over 900. The second edition (1972) was a reprinting of the original text with minor revision. The page size in the third edition is larger by 28%. Legibility is enhanced through the use of larger type. The diagrams, though enlarged, are not improved since lines are often thicker and shading denser. Nevertheless, figures are numerous and clearly drawn and labeled. Very few typographical errors were noticed in the text. A summary of the contents of each chapter at its beginning is helpful. References are all grouped at the end of the book. A systematic index, author index, and subject index all contribute to the book's usefulness.

As a review of selected aspects of the biology of intertidal animals and a description of their environment and resources, the book is a valuable addition to the library not only of marine invertebrate zoologists but of those attempting to understand, teach about, manage, or manipulate the littoral part of the coastal zone.

D. F. MALLEY

Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

(R264)

Fish and Invertebrate Culture. Water management in closed systems. *Stephen Spotte. Second edition. John Wiley & Sons, New York, N.Y. 1979. 179 p. $13.95.*

The main title of this book, Fish and Invertebrate Culture, is misleading, because the book does not contain information on rearing aquatic animals. The author admits this in the preface to the second edition. However, even the second edition does not include what might be expected from the expanded title of "Water Management in Closed Systems." The reader expects information on such things as hatchery recirculation units and rearing and holding tanks but is disappointed to find it missing. A more appropriate title for the book might be "Water Quality Management in Aquaria." This much narrower area is well covered including a good digest of the literature on the topic and valuable information from the author's extensive experience with operating large seawater aquaria. The author's statement that water quality in aquatic animal culture is a primary limiting factor is very significant, and conveying this message to the discipline where it is still notoriously neglected is the major contribution of the book.

The book emphasizes the importance of controlling environmental chemical factors in the culturing of fish in closed systems. There are nine chapters covering the major management processes (biological and mechanical filtration, physical adsorption, disinfection), gas exchange and respiration, toxicity and disease prevention, and physicochemical properties of seawater. Each chapter is followed by a brief summary of recommended management practices and hints for troubleshooting. A special chapter is devoted to analytical methods for basic water quality parameters (reactive phosphorus, ammonia, nitrite, nitrate, alkalinity, salinity, and dissolved oxygen) and recommended basic instrumentation for an aquarium laboratory.

Biological, chemical, and physical processes involved in the purification of aquarium water are adequately dealt with, but occasionally in a somewhat simplistic manner. Thus, mineralization is limited to the nitrogen cycle only (no mention of C and P), dissimilation is explained in terms of denitrification, and removal of dissolved organic carbon attributed to physical adsorption only, disregarding biochemical uptake in the aquarium itself. No mention is made of types of filter media other than gravel, for biological filtration, although a wide range of media for packed biological reactors is now available. All this is demonstrated on only one type of an aquarium system with a built-in bottom filter plate and gravel filter (design is first shown on page 45). There are also occasional errors in the mathematical equations (formulae 4, 5, and 6).

Mechanical filtration covers both gravity and rapid filters and their operation and cleaning; physical adsorption covers ion exchange and removal of nutrient ions by this mechanism, a process largely ignored in the past. Disinfection is confined to UV irradiation and ozonation. The chapter on gas exchange and respiration deals primarily with aeration by airlift pump and contains information on DO and CO_2 solubility. Useful directions for preparation of artificial seawater are given in Tables 6-2 and 6-3, but in very unusual units (major salts in kilograms per 30 240 L). Buffering capacity of aquarium water is explained in classic terms of free CO_2-bicarbonate-carbonate equilibrium, and also in terms of biological factors (respiration and nitrification).

A review of ammonia, nitrite and nitrate toxicity is presented with a critical reconsideration of the means by which ammonia becomes toxic (difference in partial pressure gradients of ammonia in the water and fish blood versus forced retention of metabolic ammonia in blood at its elevated levels in the water). Heavy metal toxicity is mentioned only briefly and recent findings on metal speciation at various pH levels are ignored.

Despite some oversimplifications and some technical shortcomings (the discrepancy with the title, and the limited experimental base), the book is a very useful source of information on its subject, based on a solid long-term practical experience. It fills a gap in fish-culture-oriented literature and it certainly deserves a place on the shelf of a practical as well as a research fish culturist.

J. BARICA AND K. R. SCOTT

Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6 (R279)

Books received/Livres reçus[1]

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Pollution Ecology of Estuarine Invertebrates. *Edited by C. W. Hart, Jr. and Samuel L. H. Fuller. Academic Press, Inc., New York, NY. 1979. xiii + 406 p. $28.50.* (This volume, one of Academic Press' Monographs on Water Pollution, contains twelve chapters by various authors on different groups of estuarine invertebrates.)

Aquatic Plants, Lake Management, and Ecosystem Consequences of Lake Harvesting. Proceedings of a Conference at Madison, Wisconsin, February 14–16, 1979. *Edited by James E. Breck, Richard T. Prentki and Orie L. Loucks. Center for Biotic Systems, Institute for Environmental Studies, University of Wisconsin, Madison, WI. 1979. 435 p.* (A limited number of free copies of this book are available upon request from: IES Communications Office, 610 Walnut Street, Room 120 WARF Bldg., Madison, WI 53706. The book contains 26 papers and a conference overview.)

***San Francisco Bay: The Urbanized Estuary. A Symposium presented during the 58th Annual Meeting of the Pacific Division, American Association for the Advancement of Science, at San Francisco State University, June 12–16, 1977.** *Edited by T. J. Conomos. Pacific Division, AAAS, c/o California Academy of Sciences, Golden Gate Park, San Francisco, CA. 1979. 493 p. $16.95.* (This volume is best described under its subtitle: "Investigations into the Natural History of San Francisco Bay and Delta With Reference to the Influence of Man." It contains 22 papers, together with a Foreword, Introduction and Summary.)

[1]Entries in this list *not* marked by an asterisk are available for review by interested readers for a 3-month period from the time of publication of the list. For information, please write to the Book Review editor.

[1]Les ouvrages inclus dans cette liste et *non* accompagnés d'un astérisque peuvent être mis à la disposition de lecteurs intéressés qui voudraient en faire l'analyse, et ce, pour une période de 3 mois à compter de la date de parution de la liste. Pour plus de renseignements, veuillez écrire au rédacteur des Comptes rendus.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Bioenergetics of stock and recruitment **D. M. Ware**

Ecology of benthic epifauna of the Estuary and Gulf of St. Lawrence: Factors influencing their distribution and abundance on buoys **P. Fradette and E. Bourget**

Influence of detritus upon colonization of stream invertebrates **B. L. Peckarsky**

Modeling growth–time and weight–length relationships in a single year-class fishery with examples from North Carolina pink and brown shrimp **M.-D. Cohen and G. S. Fishman**

Size–maturity relationships and related observations in Newfoundland populations of the lobster (*Homarus americanus*) **G. P. Ennis**

Pathogenesis of experimental vibriosis in larval American oysters, *Crassostrea virginica* **R. Elston and L. Leibovitz**

Assessment of thermal discharges on zooplankton in Conowingo Pond, Pennsylvania **D. Mathur, T. W. Robbins, and E. J. Purdy Jr.**

Sampling emerging Chironomidae (Diptera) with submerged funnel traps in a new northern Canadian reservoir, Southern Indian Lake, Manitoba **D. M. Rosenberg, A. P. Wiens, and B. Bilyj**

Statistical comparisons of several growth curves of the von Bertalanffy type **R. K. Misra**

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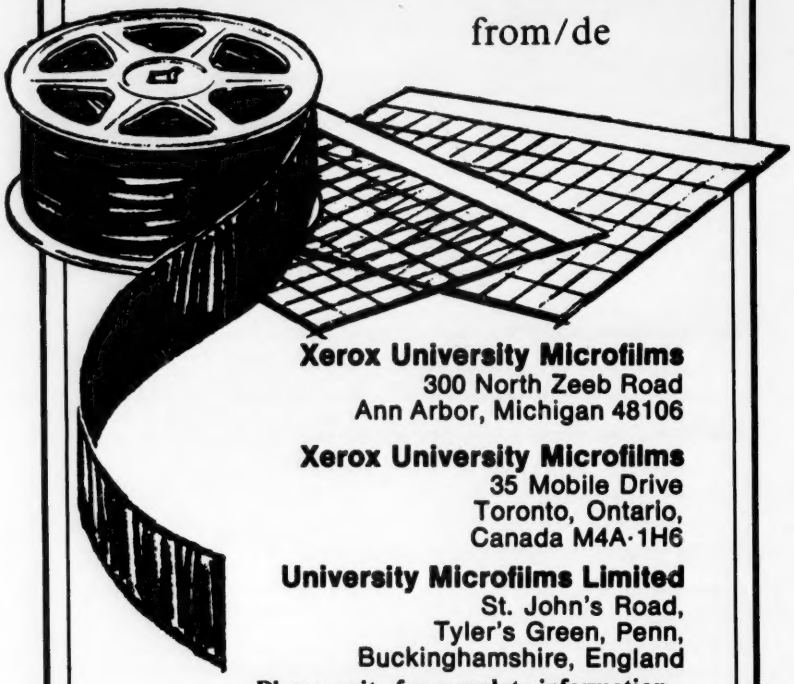

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